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Distribution of FABP7 in Neural Tissue of Socially Defeated Adult *Anolis Carolinensis*

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DISTRIBUTION OF FABP7 IN NEURAL TISSUE OF SOCIALLY DEFEATED ADULT *ANOLIS CAROLINENSIS*

by

CARMENADA LENE CAÑETE

Under the Direction of Walter Wilczynski

ABSTRACT

Due to its significance in many cellular functions, fatty acid binding protein 7 (FABP7) has become a rising topic of interest for many scientists. Immunocytochemistry was used to map the distribution of FABP7 and test whether the amount of FABP7 immunoreactivity (FABP7-IR) differed in animals that were defeated in a fight, as compared to control animals that did not engage in any social interaction. The male green anole was used as the subject because its natural tendency to establish social classes within its species provides an ideal model to observe for variation in FABP7-IR. The results showed FABP7-IR in cells and fibers of the cortex, hypothalamus, thalamus, medial preoptic area, dorsoventricular ridge, amygdala, suprachiasmatic nucleus, nucleus accumbens, nucleus rotundus, habenular area, tectum, dorsal noradrenergic and lateral forebrain bundles, and lining the third and lateral ventricles. Qualitative observation suggested higher FABP7 levels in socially defeated males than controls in all areas.

INDEX WORDS: Fatty acid binding protein 7, *Anolis carolinensis*, Subordinate, Neuroplasticity, Immunocytochemistry, Dorsomedial thalamus, Medial cortex

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DISTRIBUTION OF FABP7 IN NEURAL TISSUE OF SOCIALLY DEFEATED ADULT *ANOLIS CAROLINENSIS*

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CARMENADA LENE CAÑETE

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science

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Georgia State University

2012

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May 2012

DEDICATION

To my mother, my brother, and myself.

ACKNOWLEDGEMENTS

I would like to acknowledge Mary Karom, Georgia State University Neuroscience Institute Core Facility Supervisor, without whom I would not have been able to complete my project. Mary has given me invaluable scientific knowledge and has been one of the most significant academic guides in my graduate career. Thank you, Mary, for encouraging me to continue on my path in life as a scientist and medical professional, and for helping me to establish my position in the research world.

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1 INTRODUCTION

1.1 Animal Model

The green anole lizard, *Anolis carolinensis*, has been studied for decades for its aggressive behavior. These animals are a soundless species; however, when faced with a social situation with another animal, many different behavioral and physical changes occur to show signs of aggression (dominant lizards) or submission (subordinate lizards). This well-documented aggressive behavior is mostly observed in male lizards rather than in females, as the females are usually more receptive to an intruding female or territorial male (Wade, 2011).

Since aggressive behavior is primarily based on their reproductive season, the time of the year plays a key role in how anoles interact with one another. Spring and summer is when *A. carolinensis* reaches its peak in motivated aggressive activity, due to an increased production in gonadal steroids (Wade, 2011). During this period of the year, male anoles live separately and typically create large domains that include territories where a few females are established for mating (Lovern et al., 2004). The fall and winter seasons are when anoles are the least active. Regardless how territorial the male may be, during this time of the year they can live in groups and will show a lack of interest in almost everything that takes place around them. Their metabolism slows down, their food intake is decreased, and they may take protection together from inclement weather (Lovern et al., 2004).

Changes that occur in *A. carolinensis* during social interactions include alternating skin color from green to brown, or even black, and developing black spots behind their eyes (Baxter et al., 2001(a); Greenberg, 2002; Summers, 2001). Behavior that is considered aggressive includes these same fluctuations in skin color, physically engaging in a wrestling match with the intruder, lowering and raising the body to and from the ground in a pushup fashion (pushups), performing different head bobs directed towards the opponent, flattening the body laterally to appear larger in size (lateral compression), biting

at the neck, opening the mouth in a “gaping” fashion, and extending the skin on the neck (dewlap) (DeCourcy and Jenssen, 1994; Baxter et al., 2001(a); Greenberg, 2002; Lovern and Jenssen, 2003; Farrell and Wilczynski, 2005). Anoles can portray aggression by doing a combination of these displays, but eventually, the subordinate male will choose to not engage further in the fight and will turn brown in color, whereas the dominate lizard of the couple will remain a bright green.

Many pioneer researchers in behavioral endocrinology have discovered that androgens, such as testosterone propionate and dihydrotestosterone propionate, associated with the stress response manage the mating aggression observed in these green anoles (Adkins and Schlesinger, 1979; Greenberg et al., 1984; Morgantaler and Crews, 1978). The endocrine hypothalamic-pituitary-adrenal (HPA) axis has been found to regulate this adaptive stress response that is activated when a male anole has a social encounter with another male (Korzan and Summers, 2007; Summers and Greenberg, 1994), especially when the anoles experience stress caused by social defeat in status hierarchies (Yang and Wilczynski, 2003).

Cliff Summers (2001; 2002) and Summers et al. (2005) confirmed that once a dominance hierarchy is established between two male anoles, specific physiological changes occur in the brain that distinguish the dominant anole from the subordinate anole. A rapid increase in serotonin and glucocorticoid levels in the brain has been linked to an increase in aggressive activity in dominant anoles. The neuroendocrine reactions that socially defeated males undergo correlate to a decreased distribution of these molecules in the brain and a slower reaction in brain activity, which suggests why these male morphs do not act as aggressively towards a challenger.

All of the neurological components that support the circuitry of aggression occur in a specific pathway for *A. carolinensis* that is still being investigated today. However, according to Randy Nelson (2005), the mammalian regulation of emotion for impulsive and aggressive behaviors is generally associated with brain sites such as the hypothalamus, various areas of the cortex, hippocampus, medial preop-

tic area (MPOA), striatum, and amygdala. Other mammalian brain regions connected with stressful interactions, especially when an animal is threatened by a challenger, include the tegmentum, dorsomedial thalamic nucleus, periaqueductal area, and septum (Nelson, 2005; Gregg and Siegel, 2001). It has been discovered that the dentate gyrus in the hippocampus of mammals is also involved in these aggressive mechanisms (Kozorovitskiy and Gould, 2004).

Due to the homologous brain structures established across many other species of mammalian and even non-mammalian vertebrates, it is predicted that similar structures may be associated with the neural pathway of aggression observed in green anoles. The hormonally-regulated, highly aggressive behavior observed in this animal creates a social order (Greenberg et al., 1984; Mason and Adkins, 1976) that allows for varying psychological brain states in this species. These differing social groups offer *A. carolinensis* as a unique animal model to manipulate, explore aggression and neuromodulation, and observe for differences in regional localization of protein expression.

1.2 Protein

Fatty acid binding proteins are a family of lipid-binding proteins that have been discovered to participate in several specific functions in vertebrate tissues, including lipid molecule metabolism, fatty acid transport, cellular growth, and gene expression (Esteves and Ehrlich, 2006; Hertznel and Bernlohr, 2000; Liu et al., 2003). The seventh fatty acid binding molecule in this family is the one expressed mostly in neuronal tissue, and it can be referred to as the brain fatty acid binding protein (BFABP, B-FABP), brain lipid binding protein (BLBP), or fatty acid binding protein 7 (FABP7, FABP-7) (Denovan-Wright et al., 2000; Gerstner et al., 2008; Liu et al., 2003; Rousselot et al., 1997; Feng and Heintz, 1995). FABP7 is expressed throughout the brain tissue in regions such as the suprachiasmatic nucleus (SCN) and other areas in the hypothalamus (Gerstner et al., 2006), in radial glia and other cell receptors that line the ventricles (Feng and Heintz, 1995; Feng et al., 1994), in the hippocampus (Gerstner et al., 2008), in the cytoplasm for cellular transport (Hertznel and Bernlohr, 2000), and in many other astrocytes throughout the

brain (Gerstner et al., 2008). Recent studies even suggest that FABP7 is also involved in the proliferation of cancer cells (Slipicevic et al., 2008) and adult neurogenesis (Gerstner et al., 2008; Rousselot et al., 1997). These investigations confirm that FABP7 has been conserved throughout evolution and the ontogeny of many species, including rodents, fish, birds, and invertebrates alike (Denovan-Wright et al., 2000; Esteves and Ehrlich, 2006; Feng et al., 1994; Feng and Heintz, 1995; Gerstner et al., 2006; Gerstner et al., 2008; Hertzel and Bernlohr, 2000; Liu et al., 2003; Nelson, 2005; Rousselot et al., 1997; Slipicevic et al., 2008); therefore, it is suggested that brain tissue of reptiles may share similar expression patterns of this protein.

1.3 Purpose of the Study

Due to the link between FABP7 neuroplasticity in brain sites that are connected to areas associated with aggression, the distribution of this protein in the adult *A. carolinensis* brain was investigated. The goals of this research were to 1) determine if FABP7 is present in *A. carolinensis*, and if so, where, and 2) determine if social aggression leading to subordinate status effects the levels of FABP7-IR in green anole neural tissue.

1.4 Hypothesis

Socially defeated anoles were tested to discover if FABP7-IR would be present in the brain. It was postulated that subordinate males would show immunopositive cell labeling in brain sites such as the hypothalamus, thalamus, cortex, SCN, and amygdala area. It was also hypothesized that the control animals would produce a lower number of cells containing FABP7, as compared to those that experienced loss in a fight.

2 EXPERIMENT

2.1 Materials and Methods

All lizards used in this research study were received from Charles D. Sullivan Company (Nashville, TN) and kept in 40 liter aquaria at Georgia State University, Atlanta, GA. Each aquarium contained a PVC plastic perch, a petri dish of water, plastic leaves, and cage carpet. Procedures used were in compliance with regulations of the Institutional Animal Care and Use Committee (IACUC), under protocol #A09040. Anoles were housed either with one animal of each sex or singly (for control subjects), and in a temperature-regulated environment. Environmental conditions were aided with Repticon light bulbs and heat lamps, and maintained a range of 17.8°C – 27.8°C, 22% - 57% humidity, with a 14:10 light:dark photoperiod. All animals were mature males with an average snout to vent length (SVL) of 6.2 ± 0.2 cm, an average body weight of 4.7158 ± 0.8848 g, and an average testis weight of 0.443 ± 0.0211 g.

2.1.1 *Social behavior experimentation and tissue preservation*

Eleven male anoles were individually matched with another male, and based on how they reacted to one another, one anole became dominant and one became subordinate by the end of the trial. Ten male conspecifics were taken as control subjects for comparison. A total of 11 “fight” trials were conducted for data collection, and 32 animals were used in the experiment in total (11 dominant males, 11 subordinate males, 10 control males).

For the behavioral experiments, 2 male lizards were removed from their home cages and simultaneously introduced into a new, clean cage. These males were allowed to interact with each other for a minimum of 30 minutes and were monitored for any sign of social display of aggressive behavior. If no signs of social behavioral changes were determined after the initial 30 minutes, the males were returned to their original cages. However, if a noticeable interaction occurred within the 30 minute window, the

two anoles were left together for an additional 30 minutes (approximately 60 minutes in total), so that a clear status hierarchy developed. Any anoles that were returned to their home aquaria were left paired with their resident female. Evidence of aggression that was monitored during the fights includes skin color changes from green to brown, dark eye spots forming behind the eyes, and physical movements such as head bobs, pushups, and wrestling. Male anoles that appeared to show submission at the end of the 60 minute test were considered socially defeated and labeled as the subordinate animal of the pair, and all behavior was recorded via StopWatch Plus software. For the controls, each male was separately introduced into a new, clean cage, where he remained for the duration of the 60 minute trial.

Once the 60 minute interval was completed, each anole's SVL and body weight were documented prior to the anole being sacrificed via decapitation, and then the gonads were removed and measured. Anole heads were immersed into a 4% paraformaldehyde fixative in 0.1M phosphate-buffered solution for 18-22 hours, and then placed in 0.1 M sodium phosphate buffer (PB) for 24 hours. Subsequent dissection of all brains followed, and then the brains were saturated with a 30% sucrose solution and frozen in a 1:1 mixture of 30% sucrose in 0.1 M PB and Shandon M-1 Embedding Matrix for Frozen Sectioning (Thermo Fisher Scientific Inc., AL). Each brain specimen was frozen over dry ice in plastic microcentrifuge tubes and frozen sectioned at 25 micrometer thickness via a Leica Cryostat, and then mounted onto Fisher Scientific Superfrost Plus subbed microscope slides. The freshly sectioned tissue was immediately transferred to a -20°C storage freezer until further analysis.

Mature, control females were originally considered for comparison to the adult male dominant, subordinate, and control anoles; however, the neural tissue of all female and dominant male anoles was not directly maintained at -20°C post-sectioning. Therefore, the quality of the tissue could not be determined and those animals were not used for immunocytochemistry and subsequent analysis. The term "experimental group" in this study refers to socially defeated, subordinate male anoles.

2.1.2 Immunocytochemistry

Immunocytochemistry (ICC) was performed on the *A. carolinensis* brain sections to show where in the brain FABP7 is present. The ICC procedure was adapted from the protocol used by Dunham et al. (2009) to detect kisspeptin-IR and modified to specifically fit this project and for optimal performance. An anti-rabbit FABP7 antibody kit (Goat anti-Rabbit Elite VectaStain ABC Kit, PK-6101) with goat serum (Millipore: S20-100ml normal goat serum; Lot #: NG1731380) was used to stain the brain cells for FABP7 protein (Imgenex Corp., IMG-6611A; Lot #: 020933891B). All cells were blocked with a 10% serum and avidin solution, incubated with a 10% serum and biotin solution, and then incubated in a final step with an avidin-HRP conjugate. Lastly, the slides were stained with a 3, 3-diaminobenzidine (DAB) solution of 0.25 mg/ml concentration and dehydrated in an ethanol series with CitriSolv (Thermo Fisher Scientific Inc., AL).

2.1.3 Optimization of ICC procedure

In the initial ICC assays, the first set of slides were designated as series A (slides A1, A2, A3) and they were tested with 1:100 dilution of the FABP7 antibody; series B (slides B1, B2, B3) was used for serial dilution 1:500; series C (slides C1, C2, C3) was used for serial dilution 1:1000; series D (slides D1, D2, D3) was used for the negative control (no primary FABP7 antibody). The background staining was observed under microscopic analysis and there was no clear distinction of what cells were labeled from the ICC procedure.

The second series of ICC assays tested serial dilutions of 1:250 and 1:500 again, simultaneously. Series A was used with the FABP7 antibody at a 1:250 serial dilution. Series B was used with the FABP7 antibody at a 1:500 serial dilution. Observations at this point showed that the background staining of the whole brain tissue on slides with the 1:500 antibody dilution was not as high as it was the first time around, so any cells that demonstrated staining were more apparent. Serial dilution 1:250 for FABP7

was too dark; slides with the antibody diluted 1:500 proved to work best with the present conditions of the ICC experiment.

2.1.4 ICC control procedures

To confirm whether FABP7 antibody would bind to *A. carolinensis* brain cells, supplementary ICC trials were performed to test for ICC controls. The first several ICC experiments were done without using biotinylated secondary antibody during the ICC run in order to control for specific protein binding to the primary antibody. Then several additional assays were conducted without using any primary antibody during the ICC run to control for specific protein binding to the secondary antibody. These tests were completed to make certain that proper FABP7 antibody-protein binding took place and that the antibody was not staining cells arbitrarily. All slides with stained brain tissue were examined under the microscope for the appearance of FABP7 immunolabeling. It was confirmed that the antibody appropriately responded to the anole brain cells because no ICC-IR was detected in the tissues.

2.1.5 Microscopic observation and brain atlas

In order to understand regional localization of all the structures, an atlas was compiled to represent the *A. carolinensis* brain for identifying neural sites labeled with FABP7. Several atlases and research publications from 1953 to 2001 were used for neuroanatomical verification and nomenclature of the brain (Armstrong et al., 1953; Baxter, 2001(c); Baxter et al., 2001(a); Baxter et al., 2001(b); Greenberg, 1982; Lopez et al., 1992; Naik et al., 1981; Propper et al., 1992). The schematic diagrams were adapted from R. D. Naik et al. (1981; referred to as “Naik” in the atlas figures) and K. H. Lopez et al. (1992; referred to as “Lopez” in the atlas figures), as they produced the most complete series of rostral-caudal illustrations of the green anole brain to date. This atlas was then used for identification of neural structures and as a map to orient FABP7 immunolabeling. All figures are attached with a compiled legend, which cross checks all referenced research for consistency and nomenclature.

The anole brain sections used in this atlas includes mature female anoles with an average SVL of 5.1 ± 0.3 cm and an average body weight of 2.8654 ± 0.6930 g. All sections were cut on a coronal plane and mounted on subbed slides into 4 series, with 3 slides each, 21 sections per slide. Therefore, each section represents approximately every 100 micrometers, rostral to caudal. Some sections are slightly asymmetrical along the horizontal or vertical axis.

The antibody-stained sections came from supplemental males and females that were not utilized in this project, and they appear as brown images depicting immunoreactivity of FABP7 (referred to as “FS” images in the atlas figures; FS = FABP7 staining; see Appendix). These were matched to the adjacent violet nissl-stained sections for brain site detection (referred to as “CV” images in the atlas figures; CV = cresyl violet; see Appendix). Cresyl violet images were taken from an extra series of control male lizards that were used in this study. The FS images are not continuous throughout the spinal cord region as those sections exceeded the brain sites of interest. All sections were photographed using a Spot Imaging Solutions camera on an Olympus BX41 light microscope at 400X magnification.

2.1.6 Quantification and statistical analysis

The dorsomedial thalamus (DMT) and medial cortex (MCTX) were the brain sites chosen for quantification and statistical analysis as they contained cell bodies that were the easiest to identify. Cell body counts in these regions from the subordinate males were compared to the control animals. The photographed FS images were used to count the number of labeled cells in each section and t-tests and graphs were calculated using SPSS 12.0 for Windows and SigmaPlot 10.0 for Windows (Build 10.0.0.54). Only subordinate males that were used to count FABP7 immunolabeling in both brain regions were included in an additional analysis of one behavioral display compared to the DMT and MCTX mean cell counts ($n = 6$ for both comparisons).

Although a total of 11 subordinate and 10 control lizards were used in this study, not every lizard had ample tissue sections available for FABP7-IR observations. For example, the third control animal

exhibited elevated protein expression in the dorsomedial thalamus that was higher than the average number of cells reported for a subordinate animal; therefore, the SPSS program labeled that cell count as an outlier so that control animal was not included in the statistical analysis for that brain region. Also, other animals were not included in the quantification or statistical analysis due to loss of tissue morphology on the microscope slides that contained the brain sites of interest (these animals are not listed in Tables 3 and 4).

3 RESULTS

3.1 Behavioral Trials

The experimental group ($n = 11$) was composed of male lizards that became submissive after being paired with another male during the fight trial. Ten additional male subjects were used for the control group ($n = 10$). The number of aggressive displays was counted and the duration of eye spots, dewlap extensions, lateral compressions, open mouths, and color changes were recorded. All data were collected using StopWatch Plus software, and observations from the physical bouts are exhibited in Tables 1 and 2. Figure 1 shows a brief description of the different types of head bobs that were observed during the fight trials (adapted from DeCourcy and Jenssen, 1994).

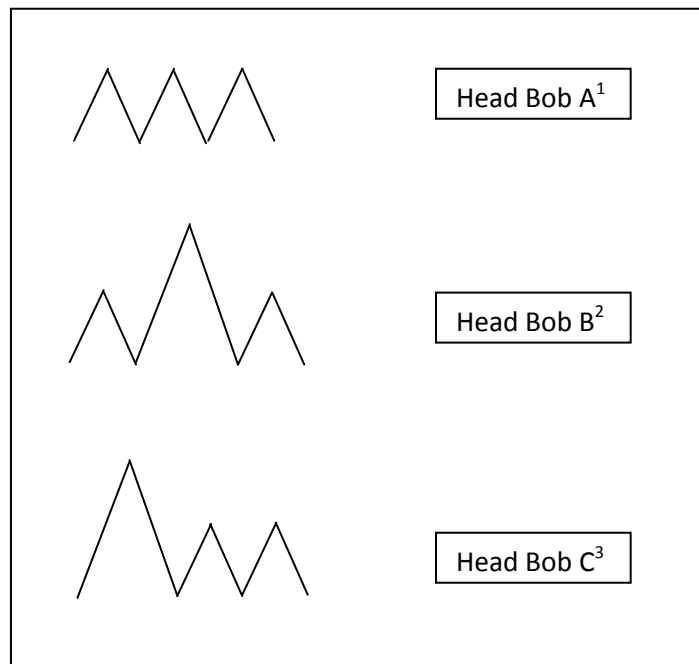


Figure 1: Diagram of Head Bobs

¹ Head Bob A = several small head nods.

² Head Bob B = several small head nods, one large head nod, then several small head nods in succession.

³ Head Bob C = one large head nod followed by several small head nods.

Table 1: StopWatch Plus Data, Fight Trials 1-6

The amount of head bobs and pushups were recorded. Eye spots, dewlap extensions, lateral compressions, open mouths, and color changes were all recorded if the action occurred. Color changes noted ranged from green to brown to black.

Sub♂ = subordinate male; ranked in numerical order: sub♂ 1 fought with dom♂ 1, etc.

Dom♂ = dominant male; ranked in numerical order.

*This animal was not used in quantification as its morphology was not maintained on the microscope slide.

**Although aggressive activity is elevated in this animal, he became submissive at the conclusion of the trial and was considered as a socially defeated subordinate in statistical analyses and protein quantification.

Anole ID	Head Bob A	Head Bob B	Head Bob C	Push-ups	Eye Spot	Dewlap Extension	Lateral Compression	Open Mouth	Color Change
Sub♂ 1	18	0	0	1	Yes	Yes	Yes	Yes	Yes
Dom♂ 1	25	12	8	0	Yes	Yes	Yes	Yes	Yes
Sub♂ 2*	1	0	0	0	No	No	No	Yes	Yes
Dom♂ 2	0	0	0	0	Yes	Yes	Yes	Yes	Yes
Sub♂ 3	5	8	8	3	No	Yes	Yes	Yes	Yes
Dom♂ 3	6	33	22	3	Yes	Yes	Yes	Yes	No
Sub♂ 4	12	0	8	0	No	No	No	No	Yes
Dom♂ 4	28	70	36	4	Yes	Yes	Yes	Yes	Yes
Sub♂ 5**	5	48	10	26	Yes	Yes	Yes	Yes	Yes
Dom♂ 5	6	8	9	3	Yes	Yes	Yes	Yes	Yes
Sub♂ 6	13	39	24	26	Yes	Yes	Yes	Yes	Yes
Dom♂ 6	8	26	34	34	Yes	Yes	Yes	Yes	Yes

Table 2: Stopwatch Plus Data, Fight Trials 7-11

The amount of head bobs and pushups were recorded. Eye spots, dewlap extensions, lateral compressions, open mouths, and color changes were all recorded if the action occurred. Color changes noted ranged from green to brown to black.

Sub♂ = subordinate male; ranked in numerical order: sub♂ 7 fought with dom♂ 7, etc.

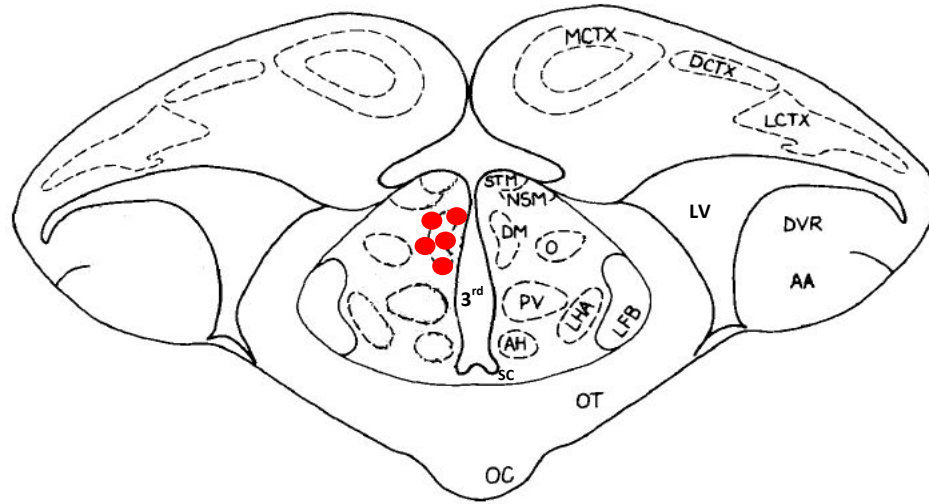
Dom♂ = dominant male; ranked in numerical order.

Anole ID	Head Bob A	Head Bob B	Head Bob C	Push-ups	Eye Spot	Dewlap Extension	Lateral Compression	Open Mouth	Color Change
Sub♂ 7	3	19	11	9	Yes	Yes	Yes	Yes	Yes
Dom♂ 7	5	41	2	37	Yes	Yes	Yes	Yes	Yes
Sub♂ 8	11	18	5	11	Yes	Yes	Yes	Yes	Yes
Dom♂ 8	38	32	37	44	Yes	Yes	Yes	Yes	Yes
Sub♂ 9	2	6	3	6	Yes	Yes	Yes	Yes	Yes
Dom♂ 9	13	16	19	31	Yes	Yes	Yes	Yes	Yes
Sub♂ 10	20	18	17	3	Yes	Yes	Yes	Yes	Yes
Dom♂ 10	23	51	16	28	Yes	Yes	Yes	Yes	Yes
Sub♂ 11	32	17	8	3	Yes	Yes	Yes	Yes	Yes
Dom♂ 11	38	31	26	34	Yes	Yes	Yes	Yes	Yes

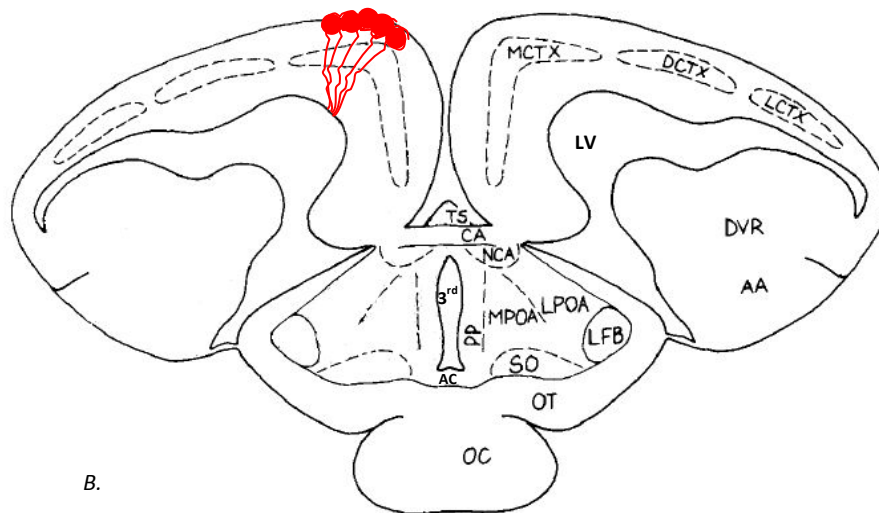
3.2 Immunocytochemistry and Microscopic Observation

All experimental and control animals (n = 11 and n = 10, respectively) were stained with the FABP7 antibody at a dilution factor of 1:500 for optimal immunolabeling. Microscopic examination showed the subordinate tissue stained with cells that appear to be radial glia, other astrocytes, ependymal cells, and single neuronal cell bodies in the third and lateral ventricular linings, medial cortex, lateral cortex, dorsal cortex, paraventricular and periventricular hypothalamic regions, dorsomedial thalamic nucleus area, MPOA, DVR, amygdala, SCN, nucleus accumbens, nucleus rotundus, habenular area, tectum, dorsal noradrenergic bundle, and the lateral forebrain bundle. Overall, the control subjects did not show as much immunoreactivity as did the socially defeated animals. Some control animals did, however, have an elevated amount of labeled cells, but not to the same degree as the subordinates.

The dorsomedial thalamus (DMT) and medial cortex (MCTX) contained the most noticeable stained cell bodies and were the brain regions chosen for quantification and statistical analysis. Figure 2 schematically demonstrates the observed FABP7-IR in these two regions. The location and shape of the stained cells noted in the diagram indicates how they appeared in the tissue sections. Figure 3 contains digital images of FABP7-IR at 400X magnification; immunolabeling seen as indicated in the dorsomedial thalamus and medial cortex regions. Tables 3 and 4 list each animal (subordinates and controls) that was used to calculate antibody-tagged cells, and the amount of cells that were counted for each animal is noted.



A.



B.

Figure 2: Schematic Diagram of FABP7 Immunolabeling

- A. Red dots in the DMT region illustrate FABP7 immunopositive cells.
- B. Red dots in the MCTX region illustrate FABP7 immunopositive cells. Some of the posterior cell fibers extended to the lateral ventricle.

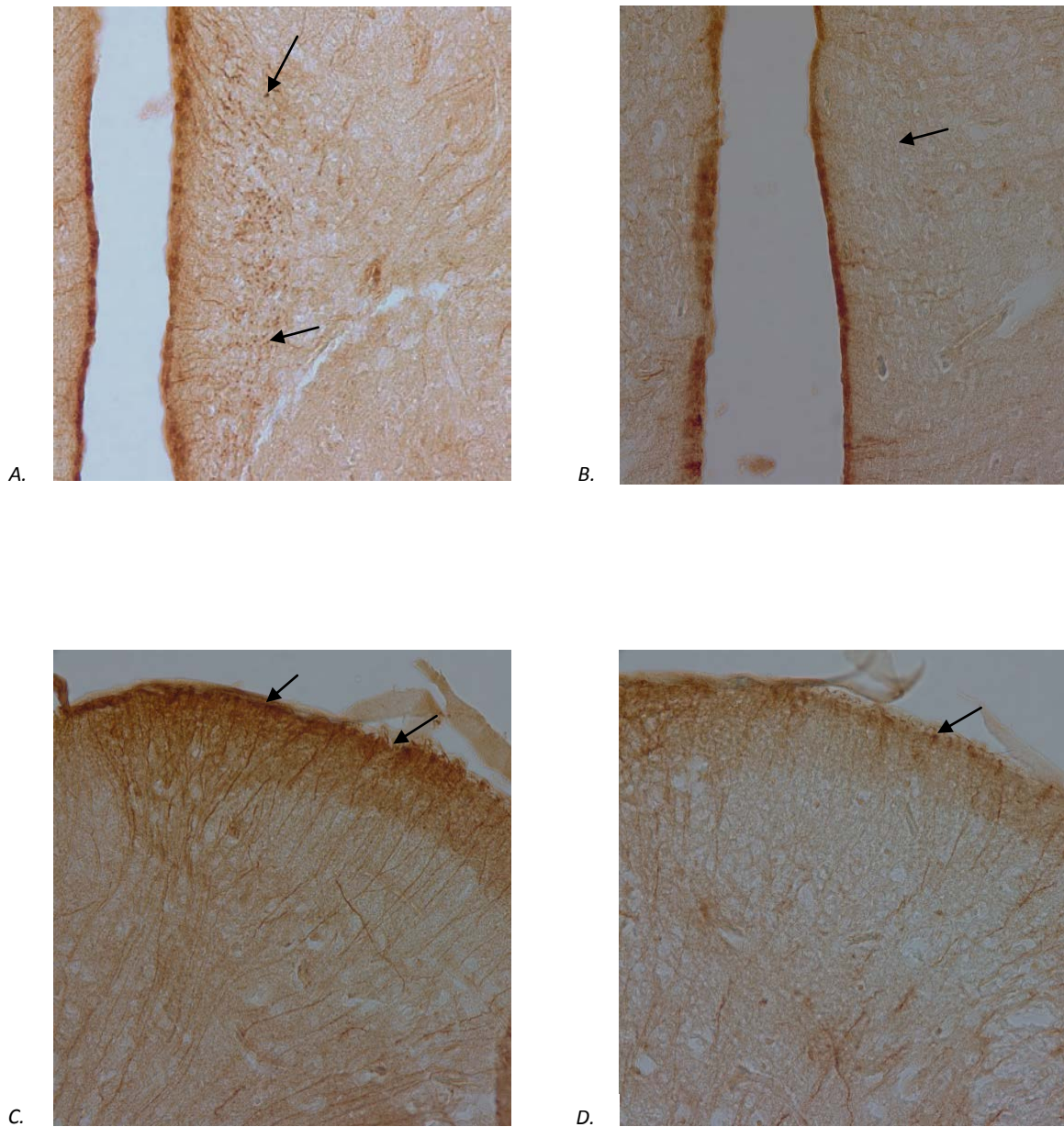


Figure 3: Example of FABP7 Immunolabeling

- A. Subordinate male anole with FABP7 antibody staining in the dorsomedial thalamic region; labeling shown as dark brown dots, depicted with arrows.
- B. Control male conspecific exemplifying a lack of FABP7 labeling in the dorsomedial thalamic region.
- C. Subordinate male anole with FABP7 antibody staining in the medial cortex; labeling shown as dark brown dots with protruding posterior fibers, depicted with arrows.
- D. Control male conspecific showing distribution of FABP7 in the medial cortex.

Table 3: Dorsomedial Thalamus Cell Counts

A total of 7 subordinate animals (n = 7) and 7 control animals (n = 7) were used in cell quantification.

*this animal was not used for quantification because the large number of immunopositive cells labeled it as an outlier, according to SPSS.

Sub♂ = subordinate male anole.

Ctrl♂ = control male anole.

Anole ID	Number of Cells	Anole ID	Number of Cells
Sub♂ 1	105	Ctrl♂ 2	18
Sub♂ 4	27	Ctrl♂ 3	166*
Sub♂ 5	47	Ctrl♂ 4	0
Sub♂ 6	38	Ctrl♂ 5	0
Sub♂ 7	57	Ctrl♂ 7	20
Sub♂ 8	69	Ctrl♂ 8	3
Sub♂ 10	130	Ctrl♂ 9	0
		Ctrl♂ 10	9

Table 4: Medial Cortex Cell Counts

A total of 8 subordinate animals (n = 8) and 10 control animals (n = 10) were used in cell quantification for this region.

Sub♂ = subordinate male anole.

Ctrl♂ = control male anole.

Anole ID	Number of Cells	Anole ID	Number of Cells
Sub♂ 1	73	Ctrl♂ 1	12
Sub♂ 5	109	Ctrl♂ 2	16
Sub♂ 6	86	Ctrl♂ 3	26
Sub♂ 7	151	Ctrl♂ 4	4
Sub♂ 8	118	Ctrl♂ 5	4
Sub♂ 9	222	Ctrl♂ 6	16
Sub♂ 10	270	Ctrl♂ 7	32
Sub♂ 11	273	Ctrl♂ 8	29
		Ctrl♂ 9	7
		Ctrl♂ 10	16

3.3 Statistical Analysis

For the dorsomedial thalamus calculations, the experimental group ($n = 7$) yielded a mean cell count of 67.667 ± 14.103 standard error (SE), and the control group ($n = 7$) yielded a mean cell count of 7.143 ± 3.284 SE. Statistical significance was reported with a 95% confidence interval for the difference in the two means ($t_{12} = 4.180$; $p = 0.001$). For the medial cortex calculations, the experimental group ($n = 8$) produced a mean cell count of 162.750 ± 28.705 SE, and the control group ($n = 10$) generated a mean cell count of 16.200 ± 3.179 SE. Statistical significance was also reported for this brain site with a 95% confidence interval for the difference in the two means ($t_{16} = 5.074$; $p = 0.001$). Comparative analysis of FABP7 expression in the two brain sites of the experimental group ($n = 7$ for DMT and $n = 8$ for MCTX) indicated statistical significance ($t_{13} = 2.830$; $p = 0.014$), whereas there was no statistical significance observed for the control group ($n = 7$ for DMT and $n = 10$ for MCTX; $t_{15} = 1.929$; $p = 0.070$). Figures 4 and 5 display this statistical data in a graph of the two separate brain regions.

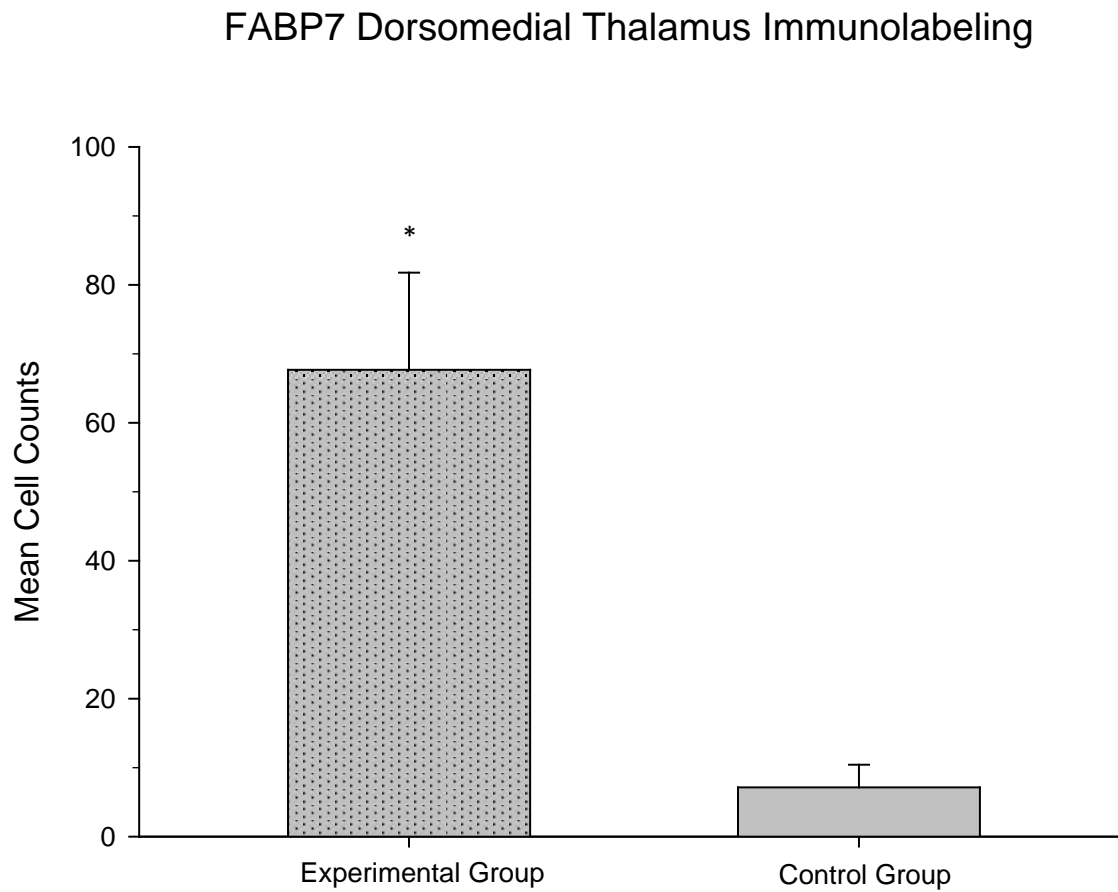


Figure 4: Statistical Graph of FABP7 Dorsomedial Thalamus Immunolabeling

Experimental group (n = 7) had a mean cell count of 67.667 ± 14.103 SE.

Control group (n = 7) had a mean cell count of 7.143 ± 3.284 SE.

* $t_{12} = 4.180$; $p = 0.001$; statistical significance was reported.

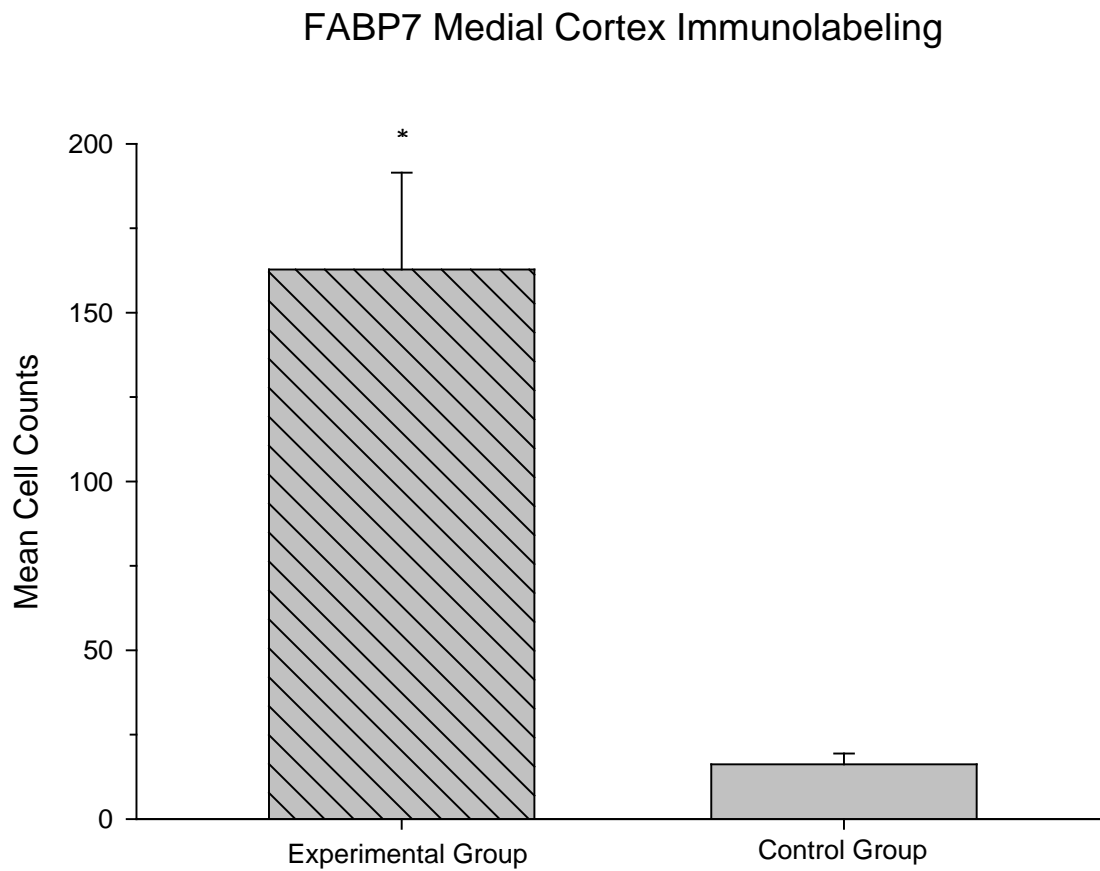


Figure 5: Statistical Graph of FABP7 Medial Cortex Immunolabeling

Experimental group (n = 8) had a mean cell count of 162.750 ± 28.705 SE.

Control group (n = 10) had a mean cell count of 16.200 ± 3.179 SE.

* $t_{16} = 5.074$; $p = 0.001$; statistical significance was reported.

4 DISCUSSION

A. carolinensis lizards have been closely studied for many years to determine what mechanisms allow them to be such an aggressive species. During the mating season, it has been proven that when male green anoles are presented with another male conspecific, a social hierarchy is established and one male will become submissive while the prevailing male will become the dominant alpha of the pair. The different social statuses allude to different neuropsychological states of subordinate and dominant anoles, which provide for an ideal model to observe for deviation in protein distribution in the brain.

Considering the immunocytochemistry results, FABP7 was found present in neural sites such as the third and lateral ventricular linings, medial cortex, lateral cortex, dorsal cortex, paraventricular and periventricular hypothalamic regions, dorsomedial thalamus, MPOA, DVR, amygdala, SCN, nucleus accumbens, nucleus rotundus, habenular area, tectum, dorsal noradrenergic bundle, and the lateral fore-brain bundle. This finding indicates that FABP7 is a functioning protein in the green anole brain during social interaction. Since previous research has shown FABP7 regionally localized in neural tissue of mammalian and non-mammalian vertebrates (Denovan-Wright et al., 2000; Feng et al., 1994; Feng and Heintz, 1995; Gerstner et al., 2006; Gerstner et al., 2008; Hertzler and Bernlohr, 2000; Liu et al., 2003; Nelson, 2005; Rousselot et al., 1997; Slipicevic et al., 2008), as well as invertebrates (Esteves and Ehrlich, 2006), green anoles can now be included in the list of species across evolution that express this protein.

After examining the quantification results, there are clear disparities in the amount of FABP7-IR observed between the control group and the experimental group. The socially defeated animals had a significantly higher frequency of FABP7 immunopositive cells in both the dorsomedial thalamus and the medial cortex (regions chosen for statistical analysis), as well as in all other areas of the brain, than did the control males. Protein distribution in the experimental animals also correlates to brain structures that have been shown to be affected by social stress and aggressive behaviors in *A. carolinensis*, such as the DVR, SCN, nucleus accumbens, amygdala, thalamus, and hypothalamus (Baxter, 2001(c); Baxter et

al., 2001(a); Baxter et al., 2001(b); Korzan and Summers, 2007). These discoveries support the hypothesis that an experience with social aggression in green anoles has an effect on FABP7-IR.

It is unclear why the socially defeated lizards contained FABP7-IR that was vastly different from the control males. Variation in immunoreactivity between the two groups of anoles could have been caused by many different variables in the behavioral trials. Having an encounter with a nearby male conspecific while being motivated by mating aggression could have been enough to activate aggressive brain regions and initiate FABP7 activity in the neural tissue of the experimental animals. FABP7 could also have been activated due to the social state the subordinate males were in after experiencing loss in a fight with a conspecific. Overall, since FABP7 was significantly more abundant in anoles that exhibited behaviorally than in the controls, for instance, all socially defeated males had visual fluctuations in skin color and head bobbing displays, this data suggests that FABP7 may be involved in communicating subordinate status to a conspecific.

The HPA axis that regulates the stress response and hormonal components that drive aggressive behavior in *A. carolinensis* (Korzan and Summers, 2007; Summers and Greenberg, 1994; Yang and Wilczynski, 2003) could be investigated to determine its relation to FABP7 expression. Dominant male anoles typically behave more aggressively than subordinate male anoles, but the results from the present study do not give any direction as to how behavior relates to social status and stress. Anoles experiencing a social interaction in general are more likely to be affected by stress than control animals; however, until additional assays are performed to measure the hormones involved in these pathways, any relation between those hormones and social status, stress, and FABP7-IR is unknown. Also, since a decrease in serotonin, dopamine, and plasma glucocorticoid levels of socially defeated anoles have been connected to a decrease in aggressive activity (Baxter et al., 2001(b); Korzan and Summers, 2007; Summers, 2001; Summers et al., 2005), it seems unlikely that FABP7 distribution is based on the levels of these molecules in the brain and blood plasma.

According to Hertzell and Bernlohr (2000), FABP7's high affinity to docosahexanoic acid indicates it may be a significant protein in the process of brain development. In neural tissue of adult zebrafish, FABP7 has been localized in regions such as the periventricular gray zone of the optic tectum, implying a connection between FABP7 and neurogenesis (Denovan-Wright et al., 2000). In rodents, neurogenesis has been proposed as a major function of FABP7 due to its spatial organization in radial glia of areas such as the ventricular zone (Feng et al., 1994). Adult song birds have as well been shown to express FABP7 in radial glia of similar brain sites that indicate adult neurogenesis (Rousselot et al., 1997). The FABP7 distribution patterns observed in the current study are comparable to those noted in the previous investigations and suggest this protein may also play a role in adult neuronal differentiation and migration in *A. carolinensis*. Having such distinction in distribution between control animals and those labeled with social status in green anoles supports the possibility of FABP7 functioning in adult neurogenesis in this species.

The appearance of FABP7 in neural sites like the cerebral cortex, hypothalamus, and SCN are consistent with the regional localization of FABP7 found in research conducted by Gerstner et al. (2006; 2008). However, FABP7 expression in rodents from those studies was confirmed after their circadian rhythms were modified. Anoles used in the present study were manipulated under the same photoperiod; therefore, additional examinations would need to be carried out before any association between FABP7 in green anoles and circadian rhythms can be determined.

In the future, supplementary studies can be conducted to explore possible answers to several questions that arise from this research. First, adding dominant male anoles as a subject group would provide results that could be compared to the data collected for socially defeated males. Basic experimentation with ICC would help determine if FABP7-IR is affected by social status, or if it is social interaction that may play more of a key role in protein expression. Hormone assays could be performed to compare stress hormone levels and androgens between the dominants and subordinates. Analysis of

data collected from these assays would offer insights to any connection between FABP7 distribution and stress or social status from aggressive interactions in green anoles.

A second component of the present study that could be evaluated between alpha dominant males and subordinates is the density of an immunopositive cell body found post-ICC. Optical density (OD) of a stained cell in the same brain region of interest for both experimental groups could be measured and compared to how aggressive one animal was to the next. If there is a trend in one social group containing the more densely-stained cell bodies in a given neural region as compared to the other social group, analysis of OD could suggest an association between FABP7 and different levels of aggression.

Another variable that could be expanded on is the duration of time allotted for the behavioral trials. Instead of limiting each pair of males to 60 minutes, they could be sacrificed at different time intervals, or even left paired together for a period of days rather than minutes or hours. During the social interaction, animals could be taken every twenty minutes up to an hour, or, after the social hierarchy is established, animals could be taken every hour up to 10 hours or more. Neural tissue of the sacrificed animals could then be evaluated for any variation in FABP7 expression at those specific time intervals. Results would indicate whether shorter or longer time periods in an established social hierarchy, or during a social interaction, affect FABP7 distribution in the brain.

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5 APPENDIX - ATLAS AND DISTRIBUTION OF FABP7 IN NEURAL TISSUE OF ADULT *ANOLIS CAROLINENSIS*



5.1 Introduction

The atlas was compiled to represent the *Anolis carolinensis* brain (green Anole - lizard) for identifying neural regions labeled with FABP7 (fatty acid binding protein 7) using immunocytochemistry (ICC). Several atlases and research articles from 1953 - 2001 were used for neuroanatomical verification and nomenclature of the brain.

5.1.1 Development of the atlas

See section 2.1.5: *Microscopic observation and brain atlas*.

5.1.2 Materials and methods – animals used

See section 2.1.5: *Microscopic observation and brain atlas*.

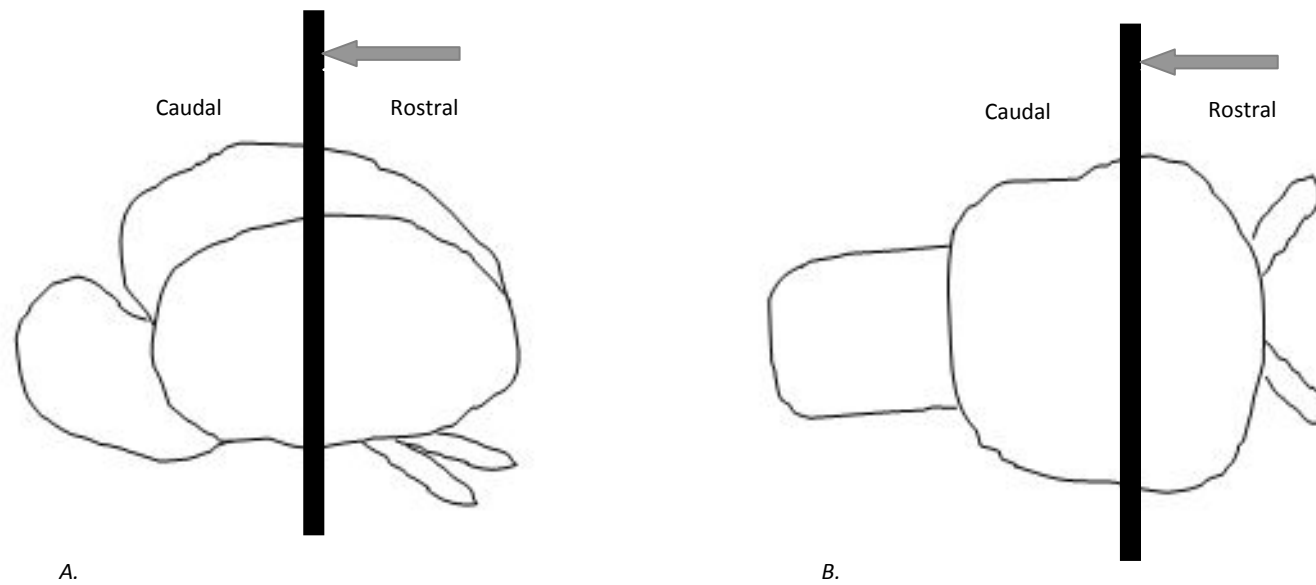


Figure 6: Lateral and Anterior View of an *Anolis carolinensis* Brain

Sections were cut on a coronal plane at 25 μ m each. A. Lateral view. B. Anterior view. Arrow indicates section cuts from rostral to caudal.

5.1.3 Cresyl violet nissl stain and ICC images

See section 2.1.5: *Microscopic observation and brain atlas*. ICC images are composed of supplemental mature male and female anole neural tissue that was not used in this study. Some cresyl violet images were taken from extra sections of male tissue from the control group used in this project.

5.1.4 Brain section information from referenced research

Although the section thickness and distance varies between scientists, the schematic illustrations are comparable using my cresyl-stained images and general morphology. The atlas was reviewed by Dr. Walter Wilczynski for validation of brain regions prior to quantification of FABP7-labeled cells.

Table 5: Summary of Section Details Used by Referenced Researchers

Primary Author	Anole Gender/Size	Section Plane	Section Thickness (μm)	Section Distance
Baxter	Adult Male 5-7 g 55-70 mm SVL	Sagittal, Coronal	25	Serial
Greenberg	Male 59-69 mm SVL	Transverse	25, 50	Alternate
Lopez	Cycling Female	Frontal	10	Serial
Naik	Male 5-9 g	Frontal, Sagittal	5, 7, 10	Serial
Propper	Adult Male & Female (Light/Dark Cycle: 14/10)	Transverse	10	Serial (every 5 th section)

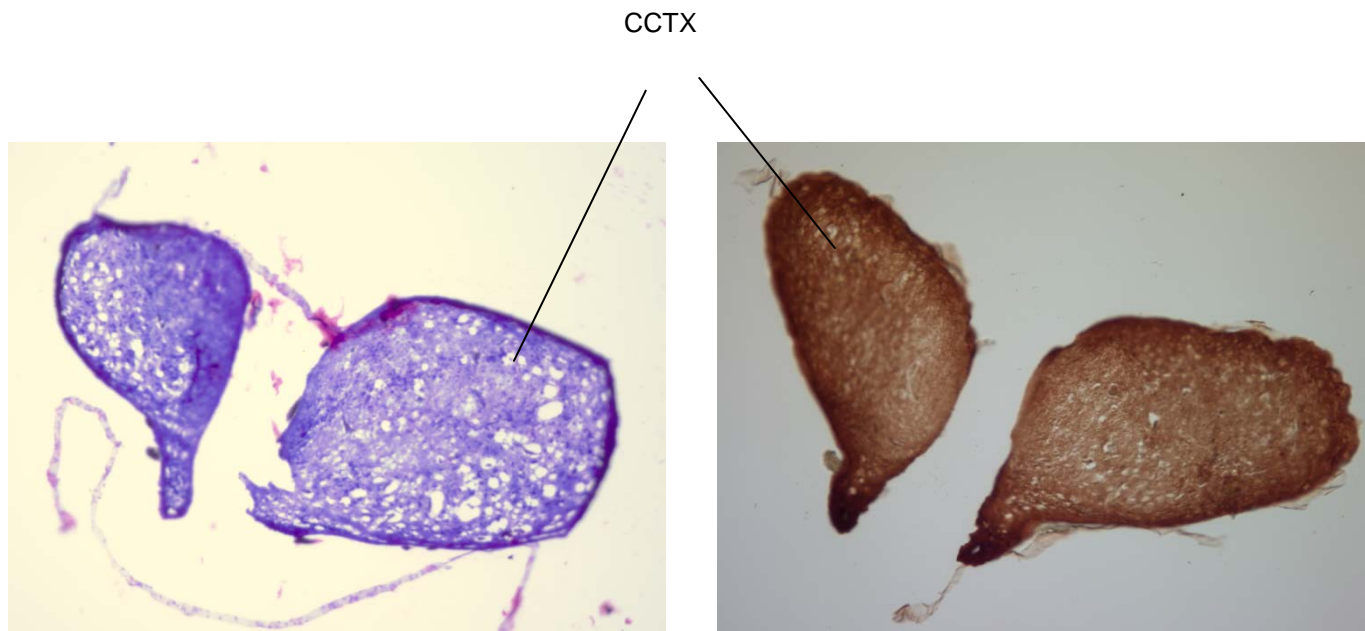
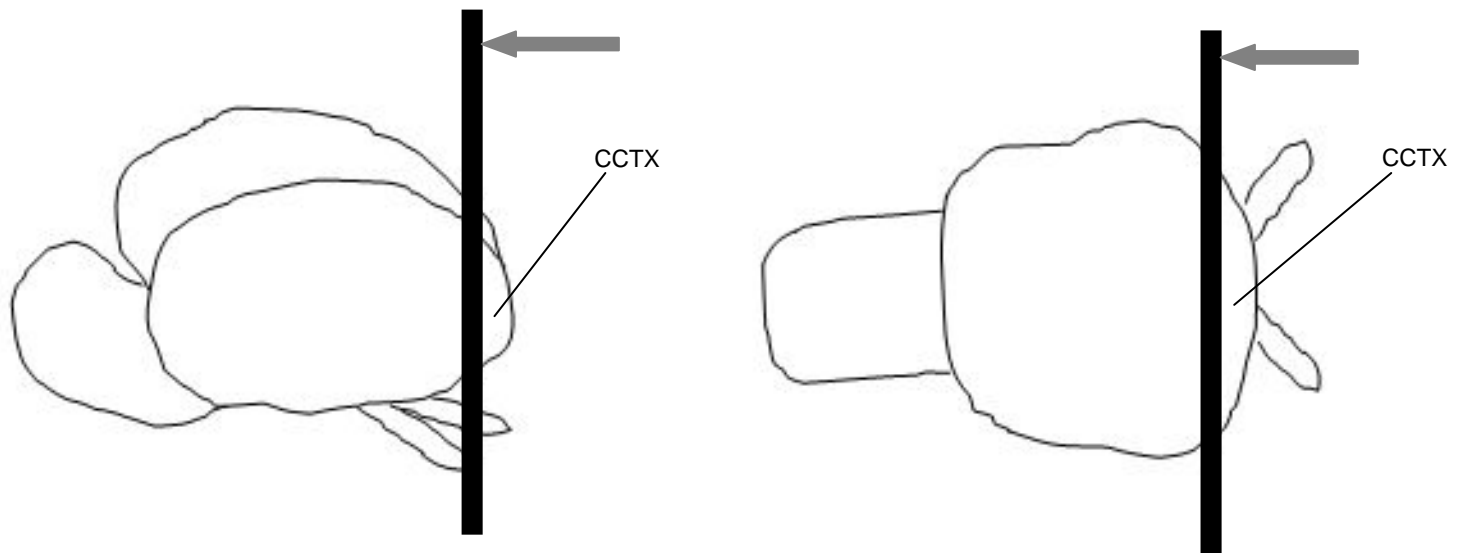


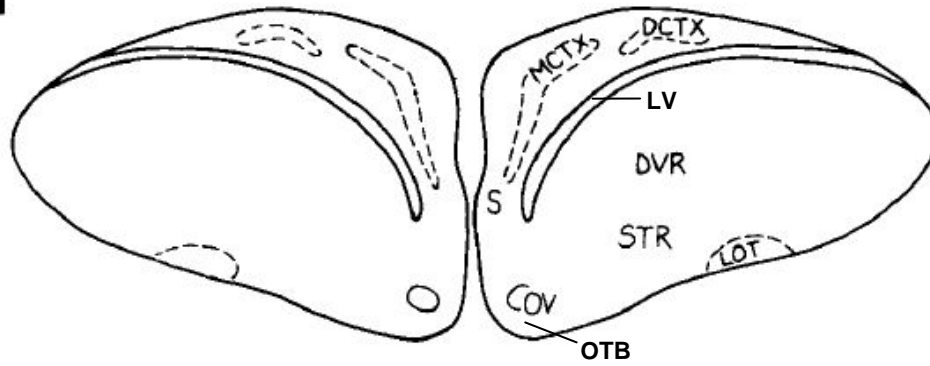
Figure 7: CV 1, FS 1

5.2 Figure 7 Legend: First section of the anole brain. Succeeding sections proceed from this point in a caudal fashion.

CCTX - cerebral cortex



1



7

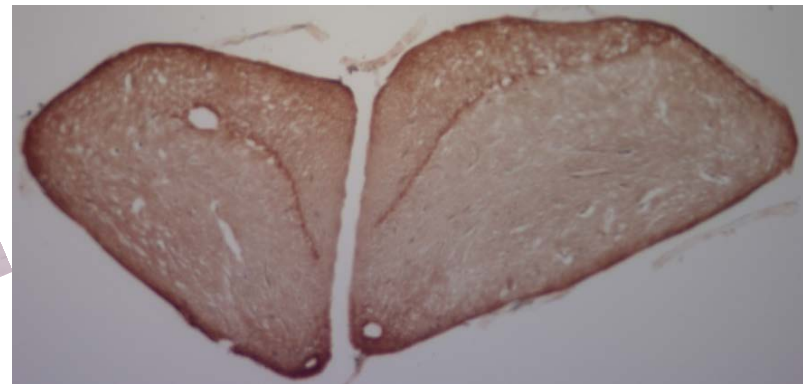
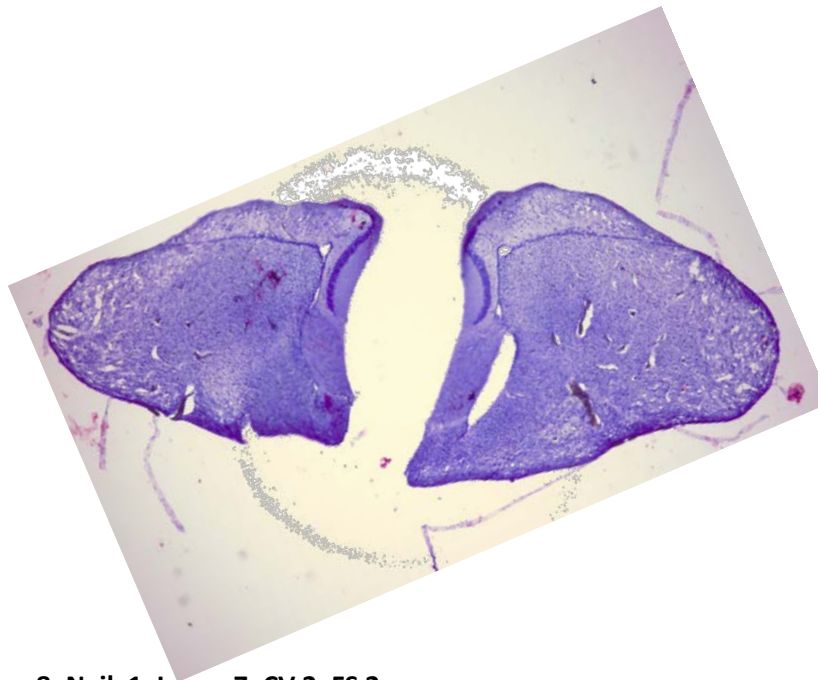
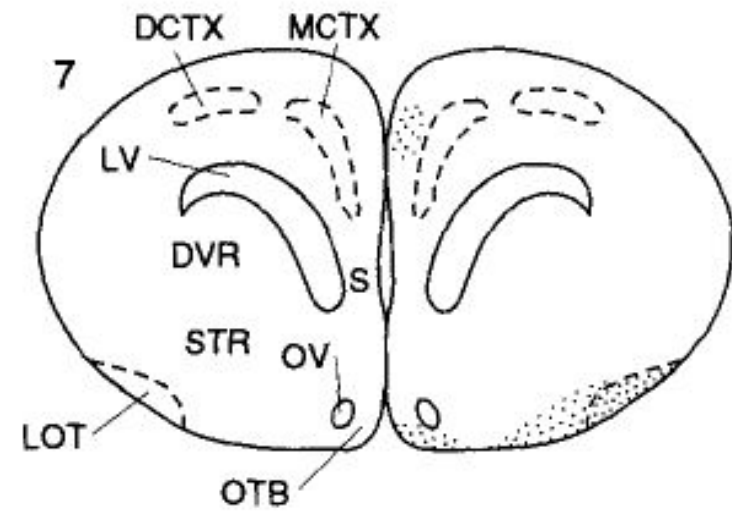


Figure 8: Naik 1, Lopez 7, CV 2, FS 2

5.3 Figure 8 Legend

DCTX - dorsal cortex

DVR - dorsal ventricular ridge

LOT - nucleus of the lateral olfactory tract

LV - lateral ventricle

MCTX - medial cortex

OTB - olfactory tubercle

OV - olfactory ventricle

S - septum

STR - striatum

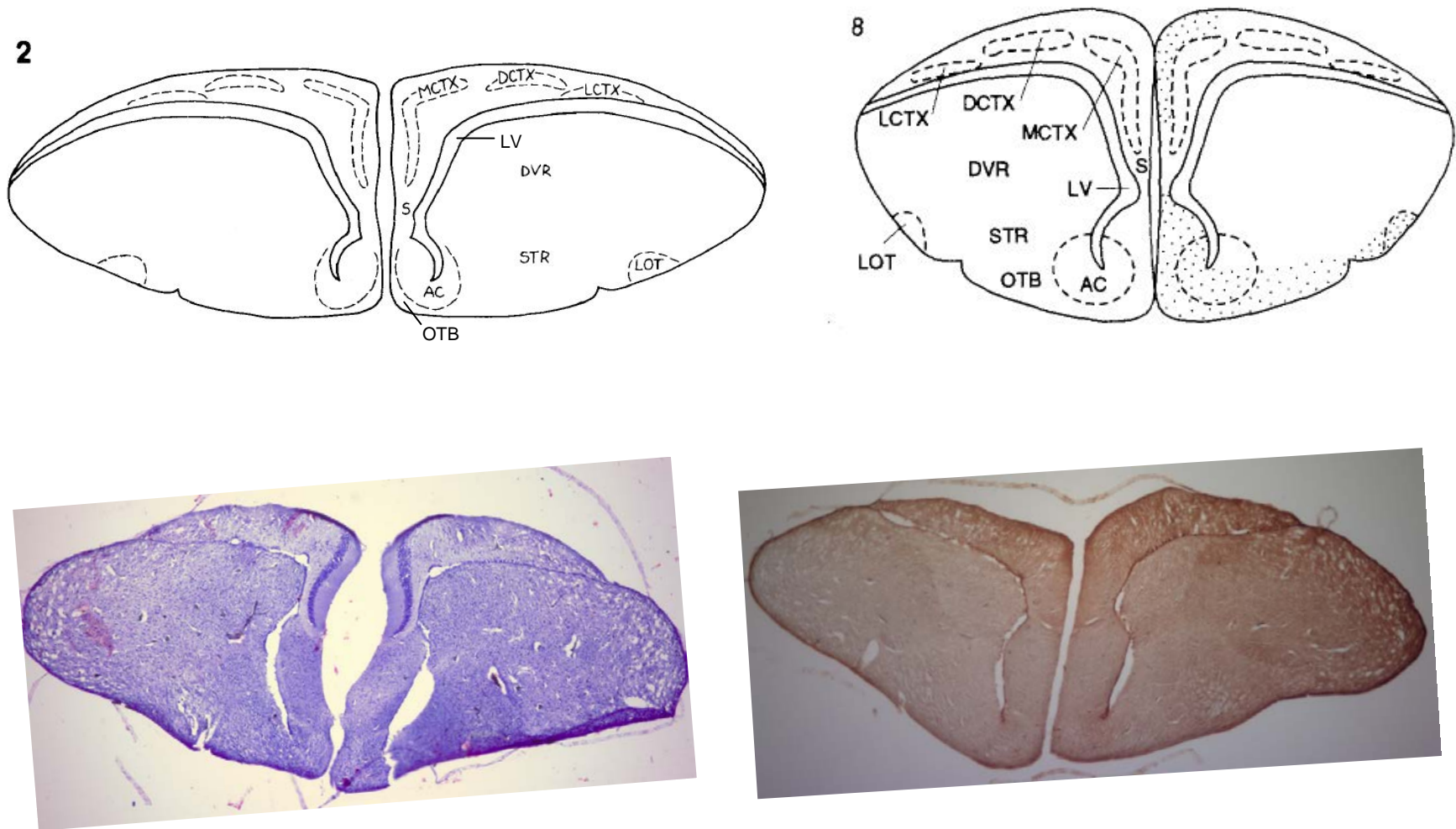


Figure 9: Naik 2, Lopez 8, CV 3, FS 3

5.4 Figure 9 Legend

AC - nucleus accumbens

DCTX - dorsal cortex

DVR - dorsal ventricular ridge

LCTX - lateral cortex

LOT - nucleus of the lateral olfactory tract

LV - lateral ventricle

MCTX - medial cortex

OTB - olfactory tubercle

S - septum

STR - striatum

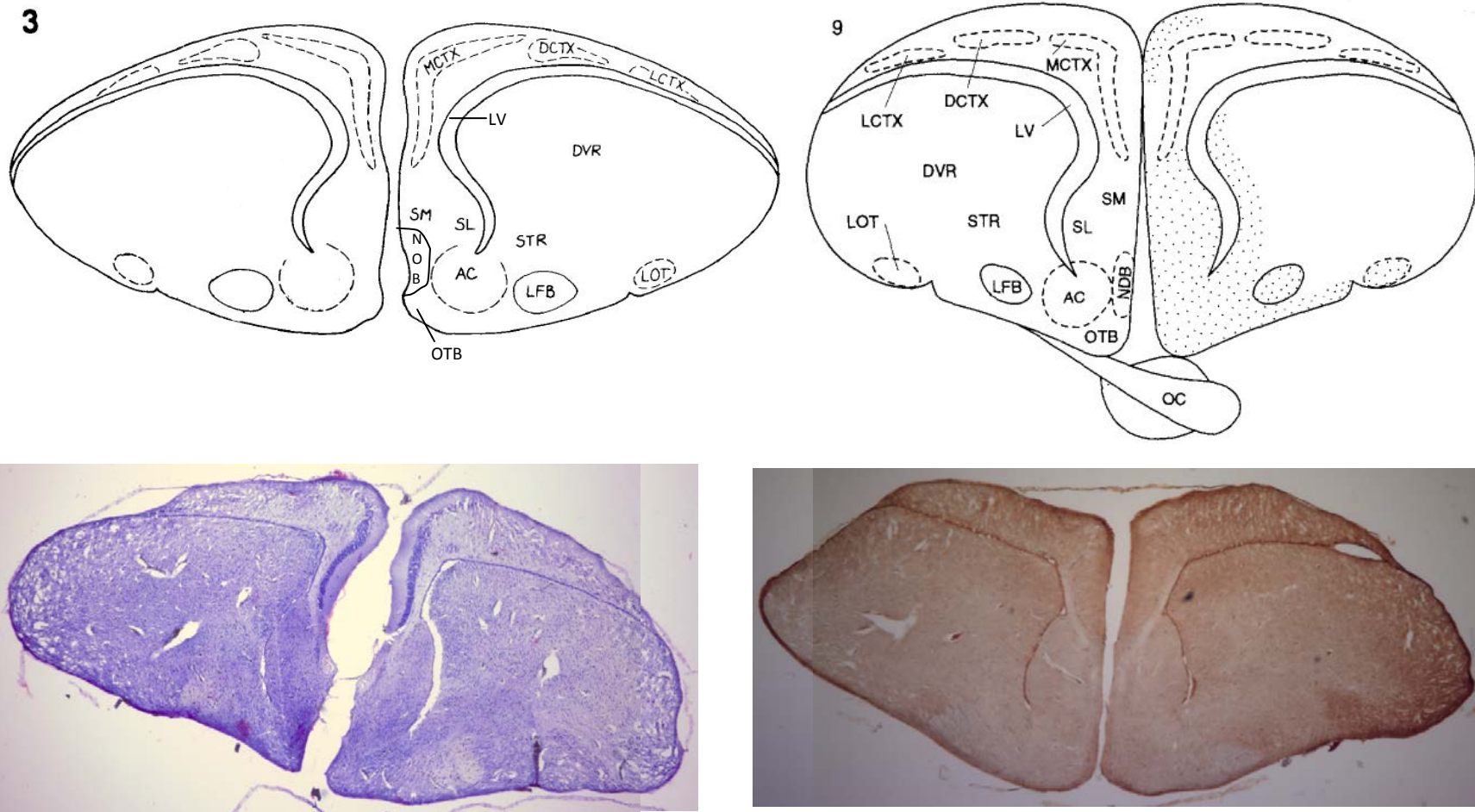


Figure 10: Naik 3, Lopez 9, CV 4, FS 4

5.5 Figure 10 Legend

AC - nucleus accumbens

DCTX - dorsal cortex

DVR - dorsal ventricular ridge

LCTX - lateral cortex

LFB - lateral forebrain bundle

LOT - nucleus of the lateral olfactory tract

LV - lateral ventricle

MCTX - medial cortex

NOB - nucleus of the diagonal band of Broca

OC - optic chiasm

OTB - olfactory tubercle

SL - lateral septal nucleus

SM - medial septal nucleus

STR - striatum

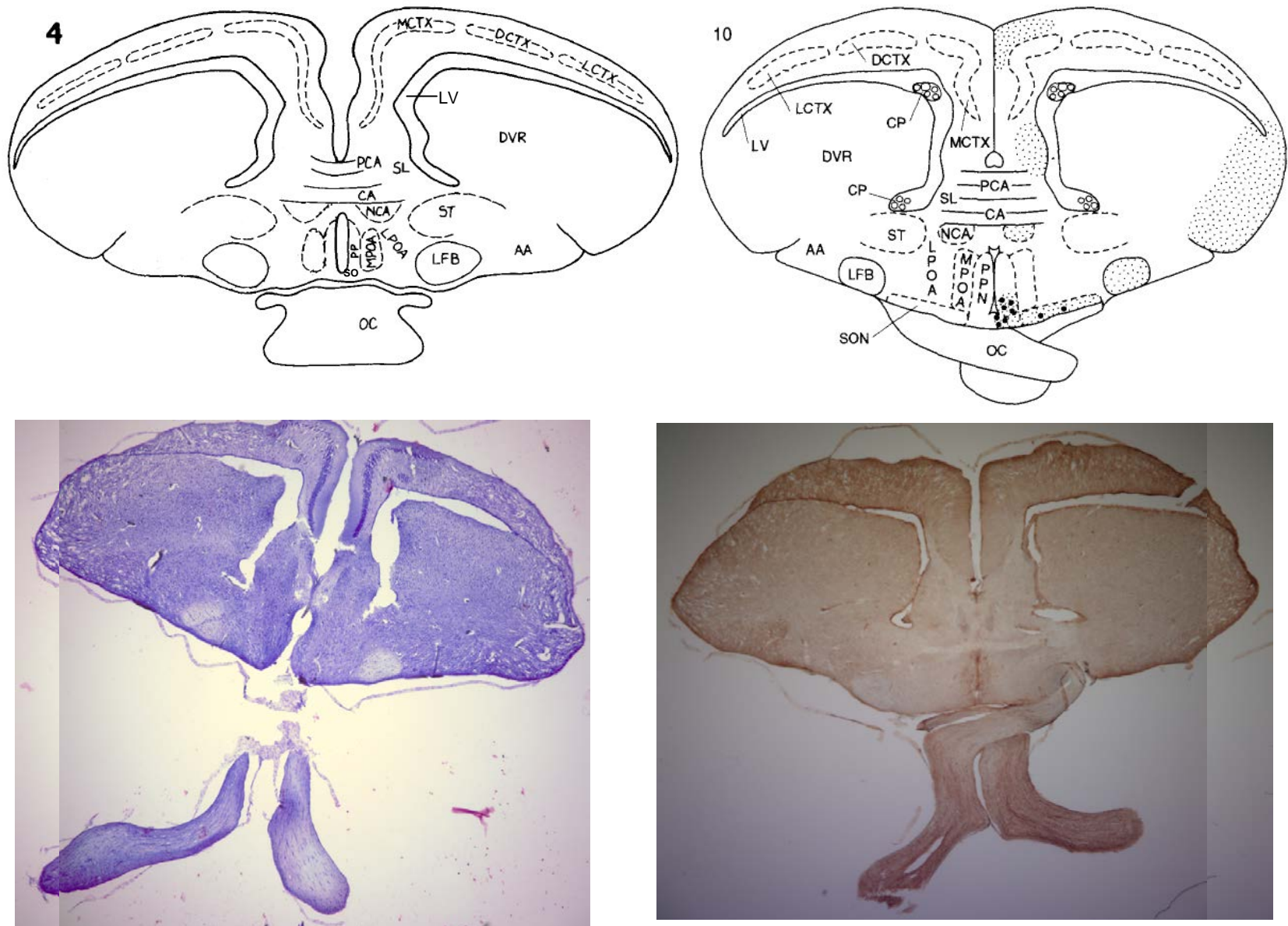


Figure 11: Naik 4, Lopez 10, CV 5, FS 5

5.6 Figure 11 Legend

AA - amygdala area

CA - anterior commissure

CP - choroid plexus

DCTX - dorsal cortex

DVR - dorsal ventricular ridge

LCTX - lateral cortex

LFB - lateral forebrain bundle

LPOA - lateral preoptic area

LV - lateral ventricle

MCTX - medial cortex

MPOA - medial preoptic area

NCA - nucleus of the anterior commissure

OC - optic chiasm

PCA - posterior commissure

PP/PPN - paraventricular preoptic nucleus

SL - lateral septal nucleus

SO/SON - supraoptic nucleus

ST - stria terminalis

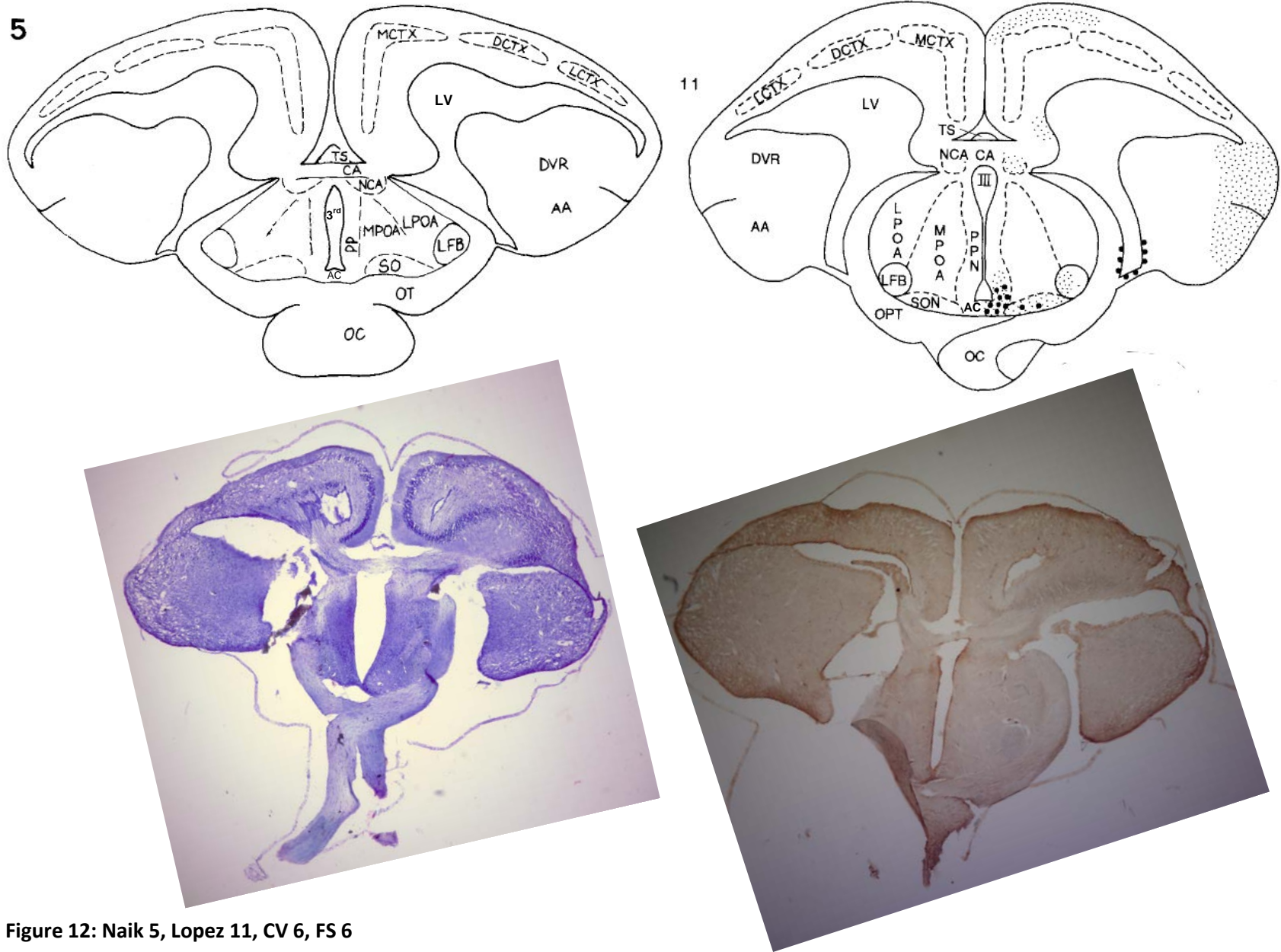


Figure 12: Naik 5, Lopez 11, CV 6, FS 6

5.7 Figure 12 Legend

AA - amygdala area

AC - nucleus accumbens

CA - anterior commissure

DCTX - dorsal cortex

DVR - dorsal ventricular ridge

LCTX - lateral cortex

LFB - lateral forebrain bundle

LPOA - lateral preoptic area

LV - lateral ventricle

MCTX - medial cortex

MPOA - medial preoptic area

NCA - nucleus of the anterior commissure

OC - optic chiasm

OPT/OT - optic tract

PP/PPN - paraventricular preoptic nucleus

SO/SON - supraoptic nucleus

TS - triangular septal nucleus

3rd/III - third ventricle

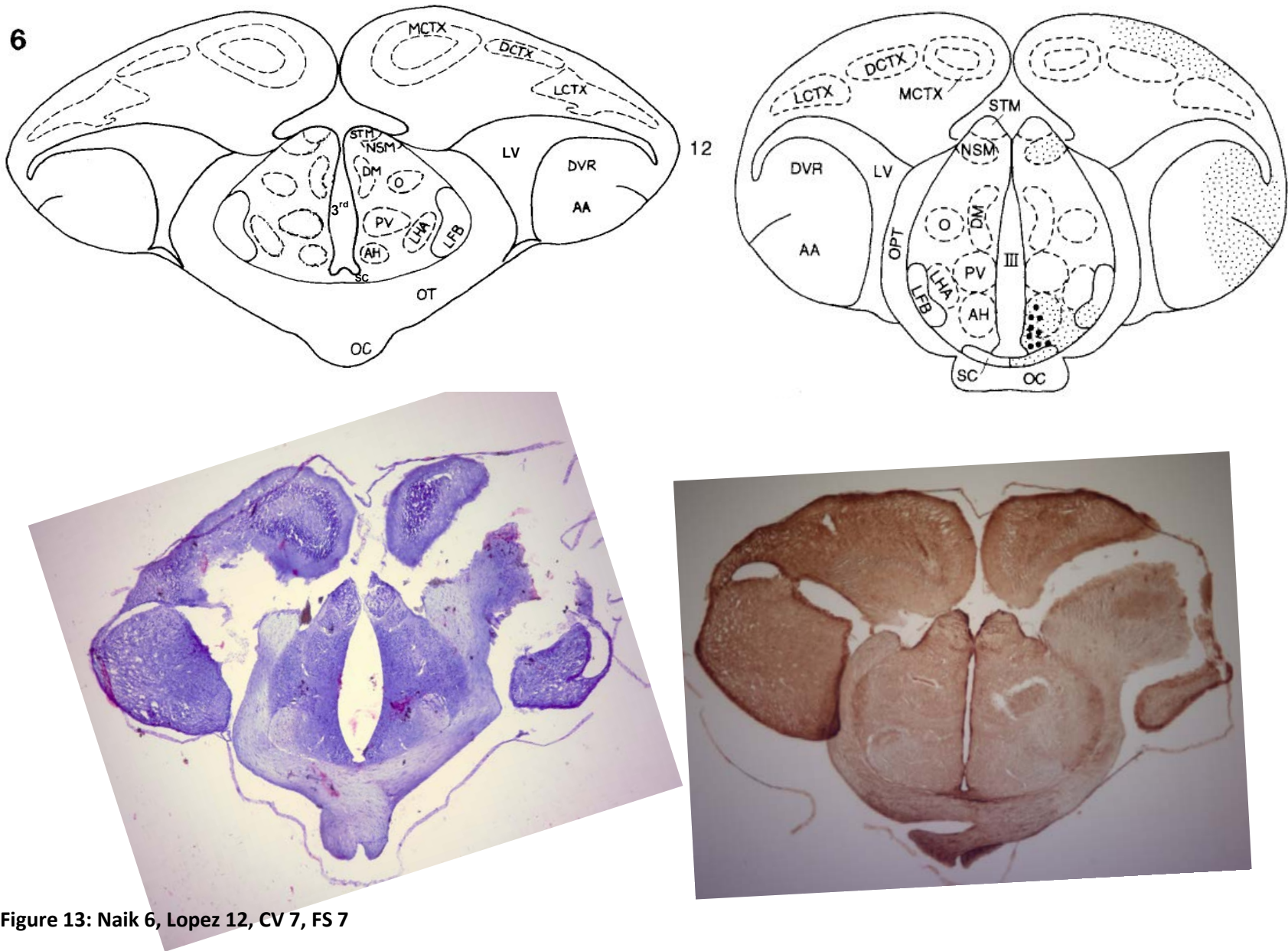


Figure 13: Naik 6, Lopez 12, CV 7, FS 7

5.8 Figure 13 Legend

AA - amygdala area

AH - anterior hypothalamic area

DCTX - dorsal cortex

DM - dorsomedial nucleus of the thalamus

DVR - dorsal ventricular ridge

LCTX - lateral cortex

LFB - lateral forebrain bundle

LHA - lateral hypothalamic area

LV - lateral ventricle

MCTX - medial cortex

NSM - nucleus of the stria medullaris

O - oval nucleus

OC - optic chiasm

OPT/OT - optic tract

PV - paraventricular nucleus

SC - suprachiasmatic nucleus

STM - stria medullaris

3rd/III - third ventricle

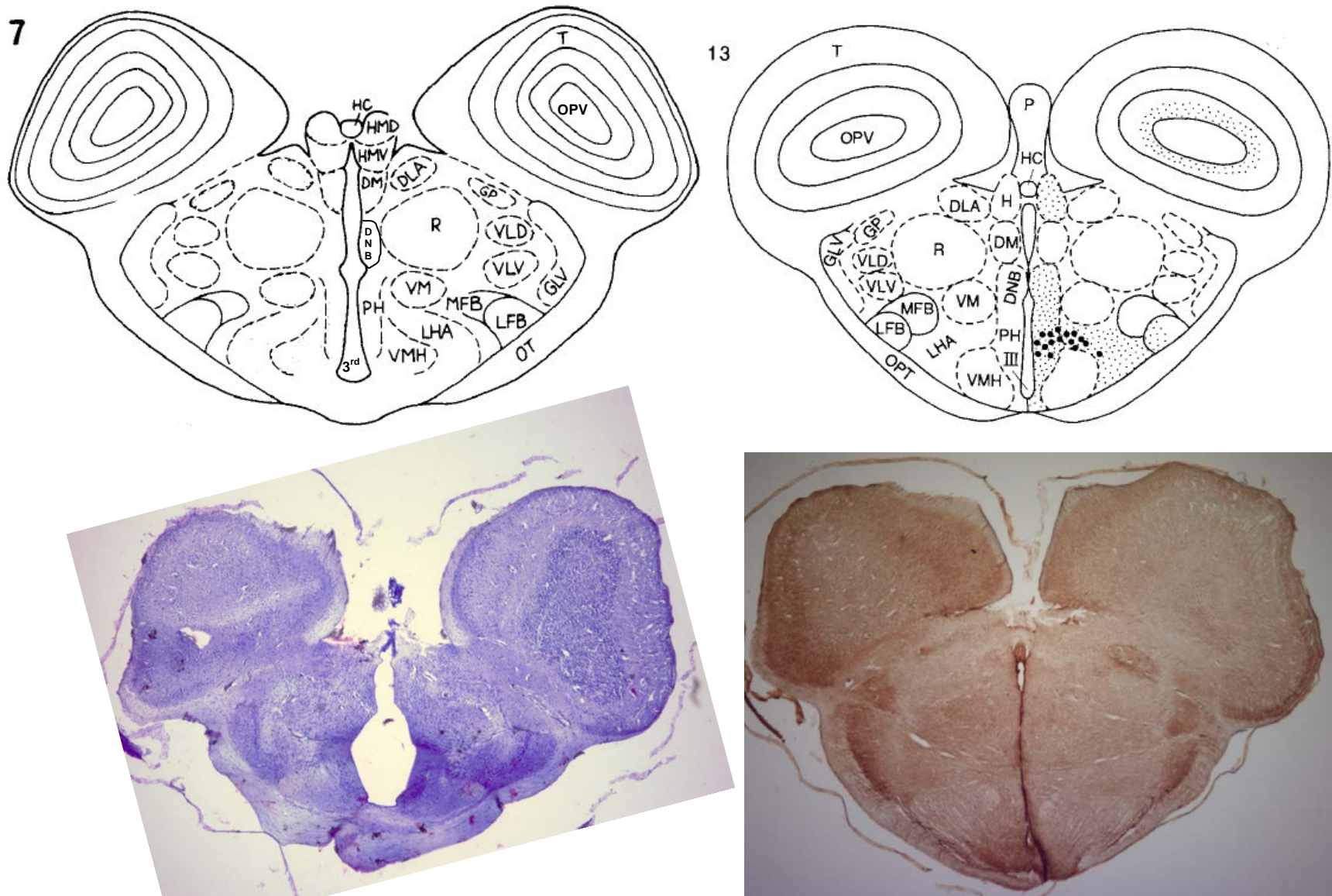


Figure 14: Naik 7, Lopez 13, CV 8, FS 8

5.9 Figure 14 Legend

DLA - anterior dorsolateral nucleus of the thalamus

DM - dorsomedial nucleus of the thalamus

DNB - dorsal noradrenergic bundle

GLV - ventrolateral geniculate nucleus

GP - pretectal geniculate nucleus

H - medial habenular nucleus

HC - habenular commissure

HMD - dorsomedial habenular nucleus

HMV - ventromedial habenular nucleus

LFB - lateral forebrain bundle

LHA - lateral hypothalamic area

MFB - medial forebrain bundle

OPT/OT - optic tract

OPV - optic ventricle

P - pars nervosa

PH - periventricular nucleus of the hypothalamus

R - nucleus rotundus

T - tectum

VLD - dorsal ventrolateral nucleus of the thalamus

VLV - ventral ventrolateral nucleus of the thalamus

VM - ventromedial nucleus of the thalamus

VMH - ventromedial nucleus of the hypothalamus

3rd/III - third ventricle

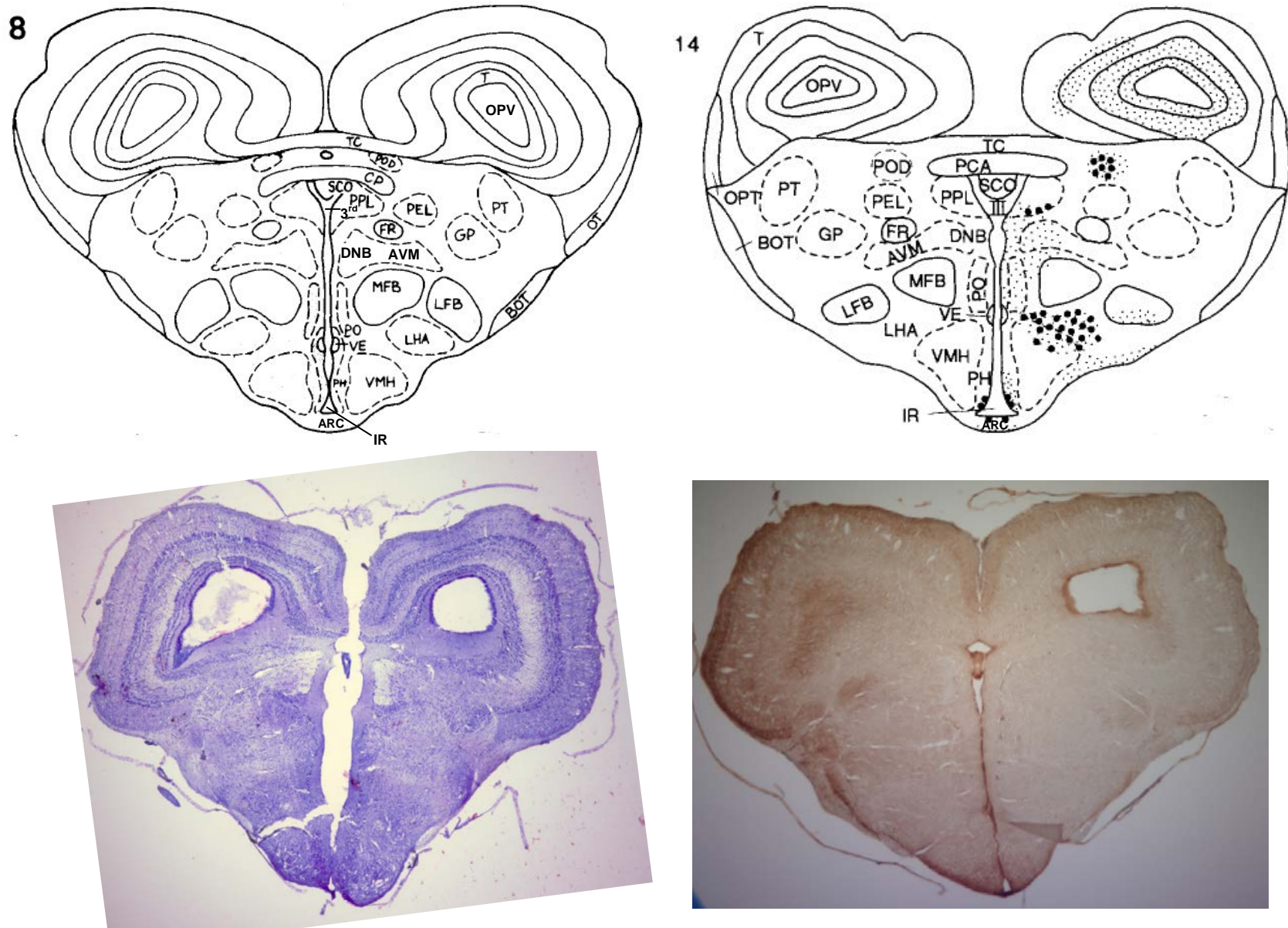


Figure 15: Naik 8, Lopez 14, CV 8, FS 8

5.10 Figure 15 Legend

ARC - arcuate nucleus

AVM - ventromedial area of thalamus

BOT - basal optic tract

CP/PCA - posterior commissure

DNB - dorsal noradrenergic bundle

FR - fasciculus retroflexus

GP - pretectal geniculate nucleus

IR - infundibular recess

LFB - lateral forebrain bundle

LHA - lateral hypothalamic area

MFB - medial forebrain bundle

OPV - optic ventricle

OPT/OT - optic tract

PEL - extended lentiform nucleus of the thalamus

PH - periventricular nucleus of the hypothalamus

PO - nucleus of the posterior hypothalamus

POD - posterodorsal nucleus

PPL - plicated lentiform nucleus of the thalamus

PT - pretectal nucleus

SCO - subcommissural organ

T - tectum

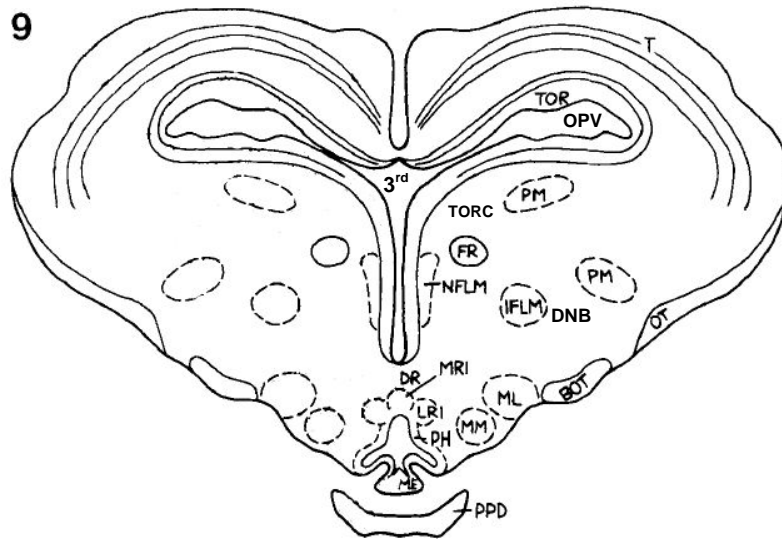
TC - tectal commissure

VE - ventricular ependymal organ

VMH - ventromedial nucleus of the hypothalamus

3rd/III - third ventricle

9



15

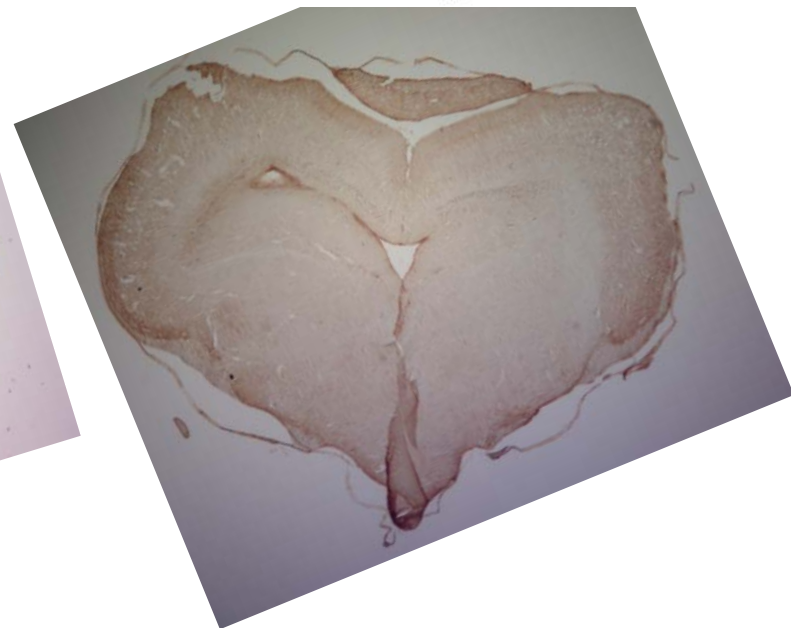
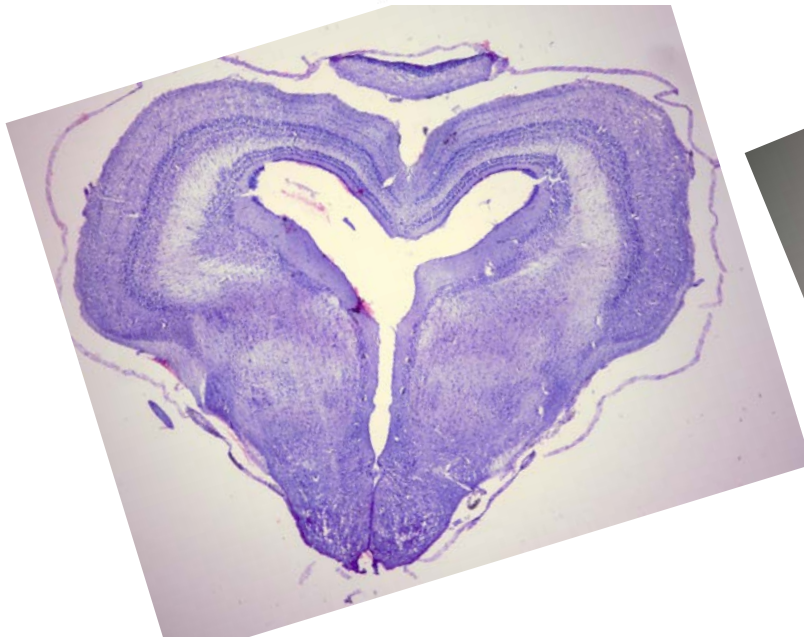
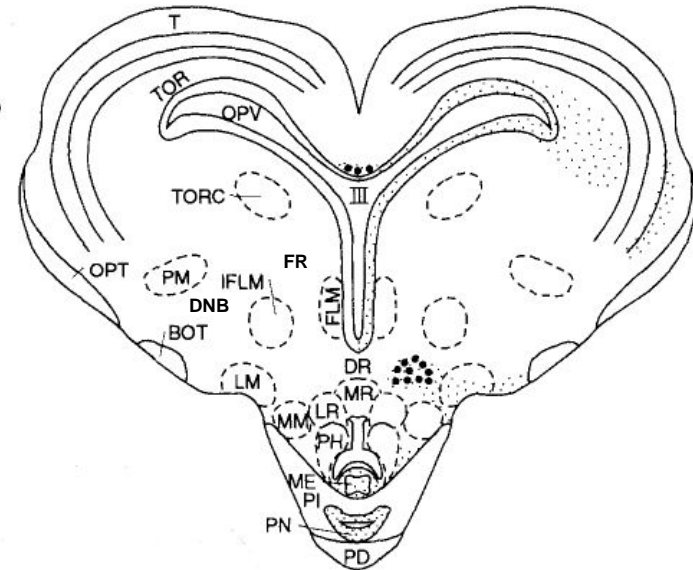


Figure 16: Naik 9, Lopez 15, CV 10, FS 10

5.11 Figure 16 Legend

BOT - basal optic tract

DNB - dorsal noradrenergic bundle

DR - retroinfundibular decussation

FLM - longitudinal medial fasciculus

FR - fasciculus retroflexus

IFLM - interstitial nucleus of the longitudinal medial
fasciculus

LM/ML - lateral mammillary nucleus

LR/LRI - lateral nucleus of the infundibular recess

ME - median eminence

MM - medial mammillary nucleus

MR/MRI - medial nucleus of the infundibular recess

NFLM - nucleus of the longitudinal medial fasciculus

OPT/OP - optic tract

OPV - optic ventricle

PD/PPD - pars distalis of the pituitary

PH - periventricular nucleus of the hypothalamus

PI - pars intermedia of the pituitary

PM - nucleus profundus of the mesencephalon

PN - pars nervosa of the pituitary

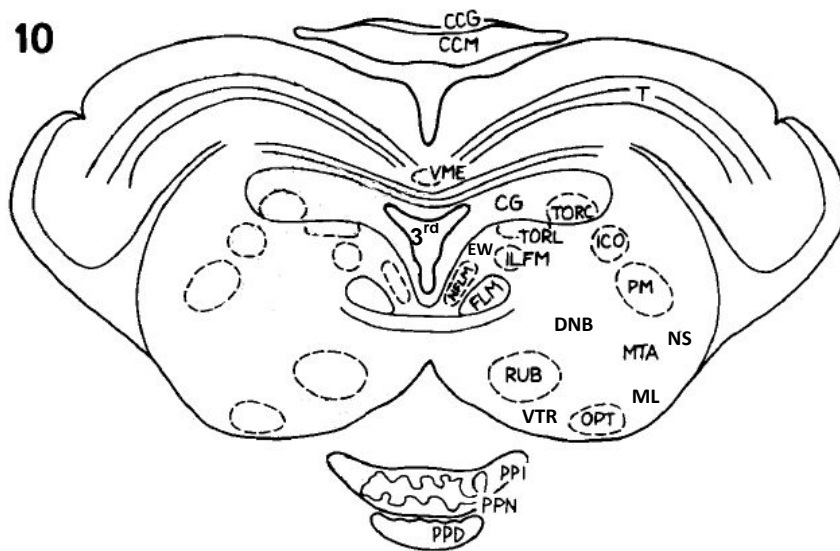
T - tectum

TOR - torus semicircularis

TORC - central nucleus of the torus semicircularis

3rd/III - third ventricle

10



16

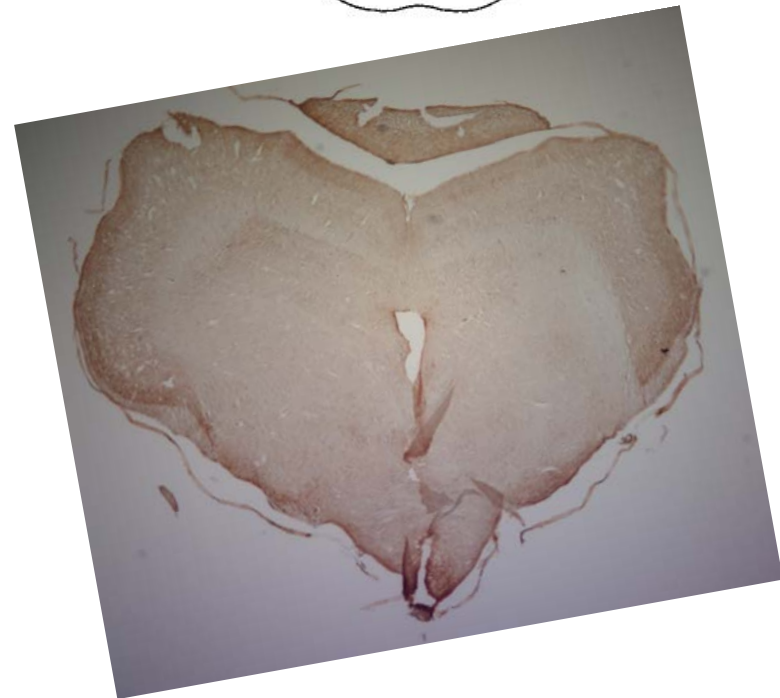
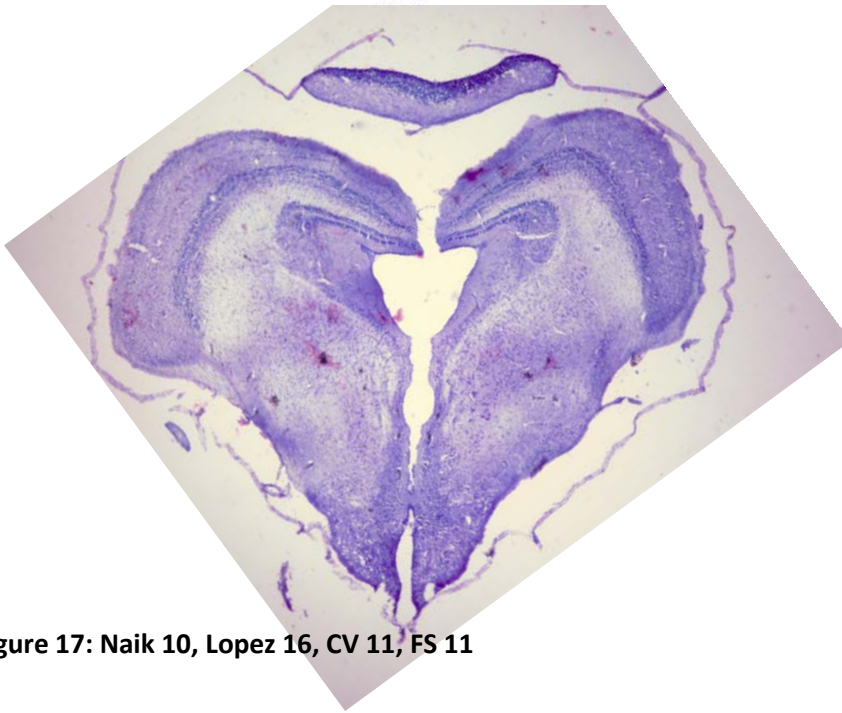
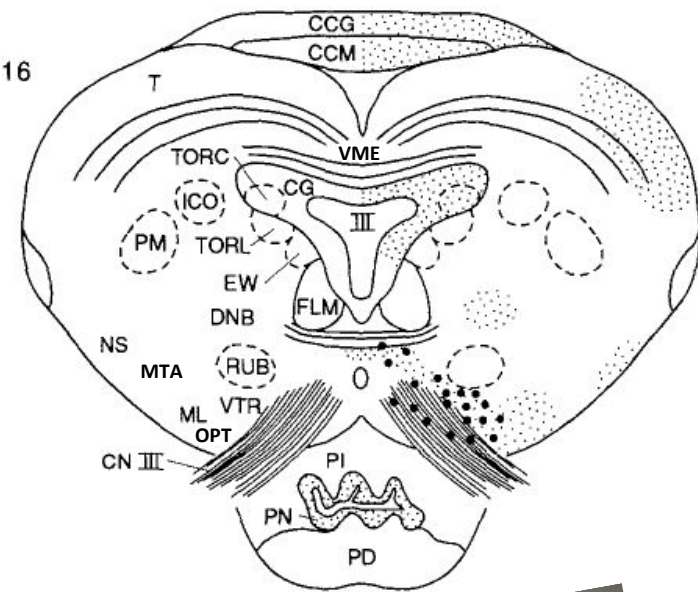


Figure 17: Naik 10, Lopez 16, CV 11, FS 11

5.12 Figure 17 Legend

CCG - granular layer of the cerebellar cortex

CCM - molecular layer of the cerebellar cortex

CG - central gray

CnIII - 3rd cranial nerve

DNB - dorsal noradrenergic bundle

EW - Edinger-Westphal nucleus

FLM - longitudinal medial fasciculus

ICO - intercollicular nucleus

ILFM - interstitial nucleus of the longitudinal medial
fasciculus

ML - lateral mammillary nucleus

MTA - mesencephalic tegmental area

NFLM - nucleus of the longitudinal medial fasciculus

NS - nigrostriatal tract

OPT - nucleus of the optic tectum

PD/PPD - pars distalis of the pituitary

PI/PPI - pars intermedia of the pituitary

PM - nucleus profundus of the mesencephalon

PN/PPN - pars nervosa of the pituitary

RUB - red nucleus

T - tectum

TORC - central nucleus of the torus semicircularis

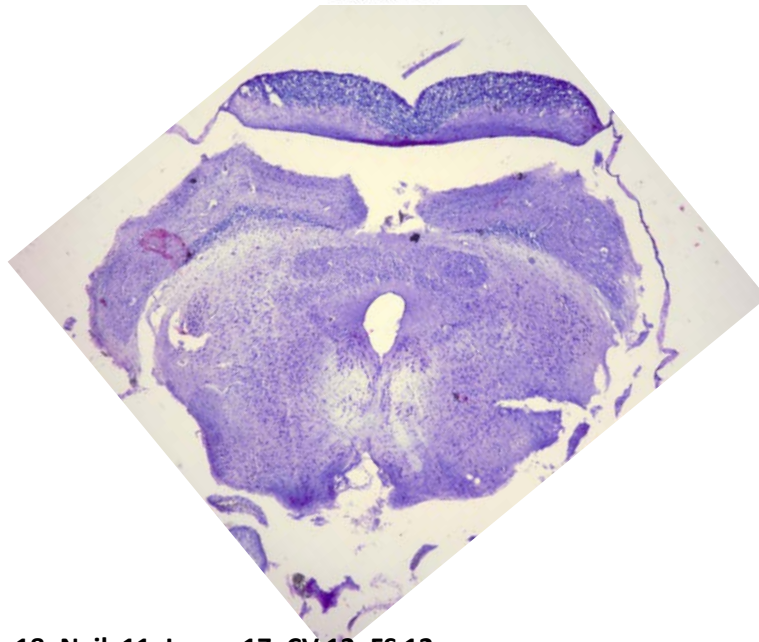
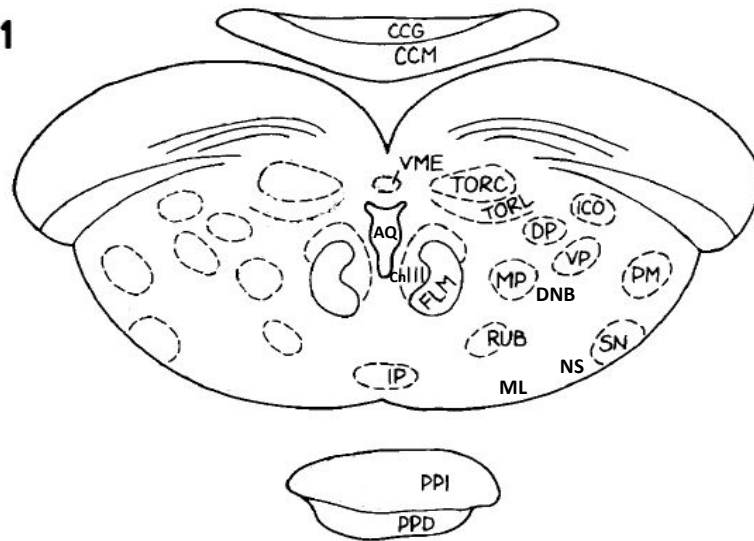
TORL - laminar nucleus of the torus semicircularis

VME - mesencephalic nucleus of the trigeminal nerve

VTR - ventral tegmental area

3rd/III - third ventricle

11



17

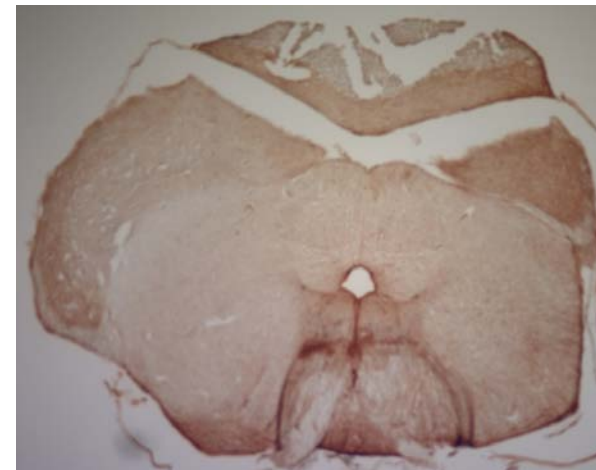
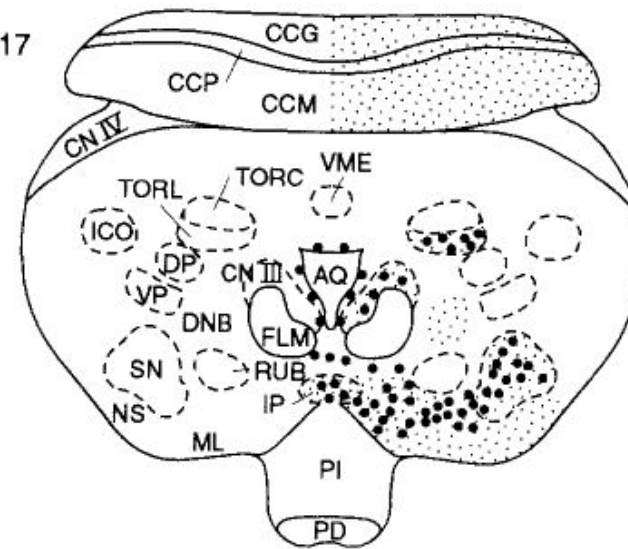


Figure 18: Naik 11, Lopez 17, CV 12, FS 12

5.13 Figure 18 Legend

AQ - cerebral aqueduct

CCG - granular layer of the cerebellar cortex

CCM - molecular layer of the cerebellar cortex

CnIII - 3rd cranial nerve

DNB - dorsal noradrenergic bundle

DP - dorsal pretectal nucleus

FLM - longitudinal medial fasciculus

ICO - intercollicular nucleus

IP - interpeduncular nucleus

ML - lateral mammillary nucleus

MP - medial pretectal nucleus

NS - nigrostriatal tract

PD/PPD - pars distalis of the pituitary

PI/PPI - pars intermedia of the pituitary

PM - nucleus profundus of the mesencephalon

RUB - red nucleus

SN - substantia nigra

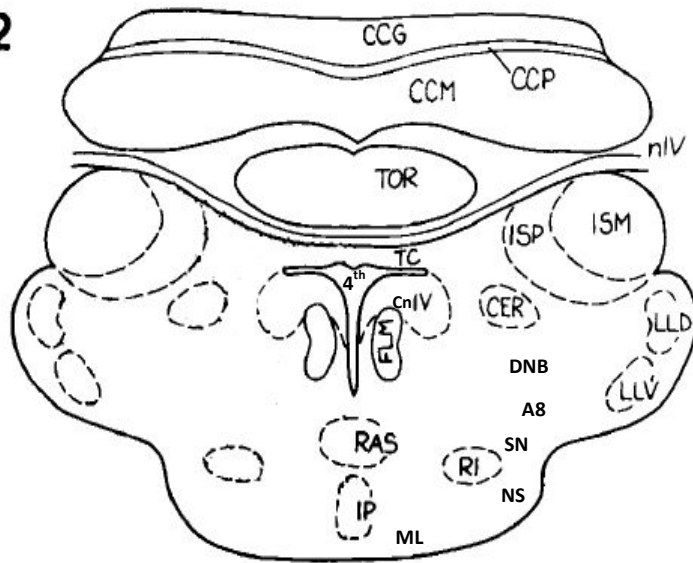
TORC - central nucleus of the torus semicircularis

TORL - laminar nucleus of the torus semicircularis

VME - mesencephalic nucleus of the trigeminal nerve

VP - ventral pretectal nucleus

12



18

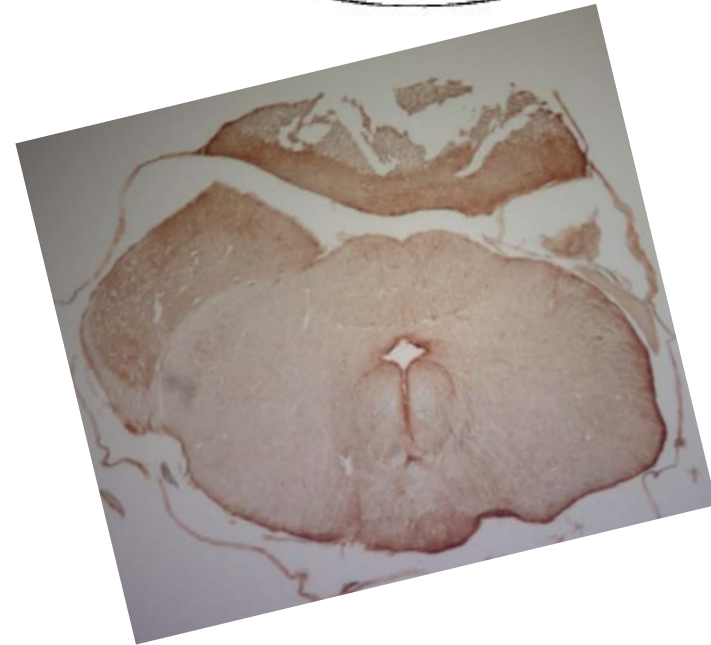
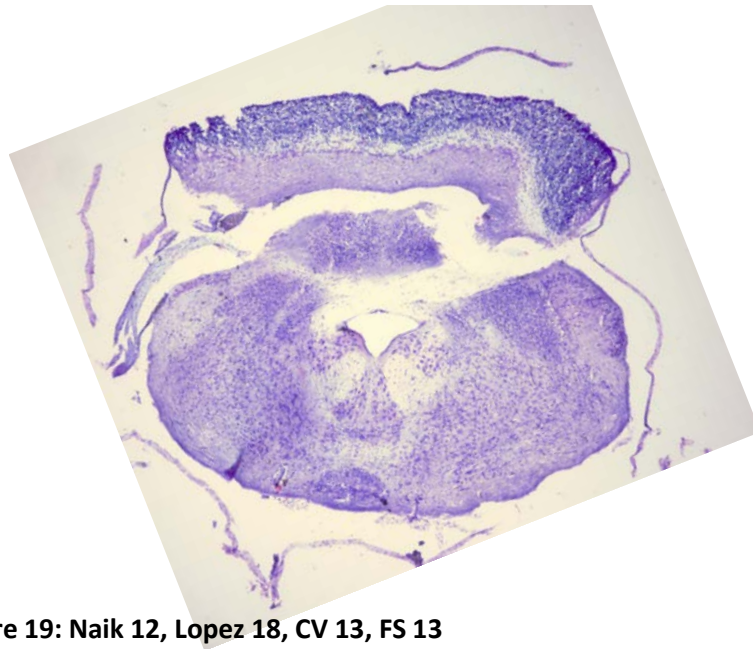
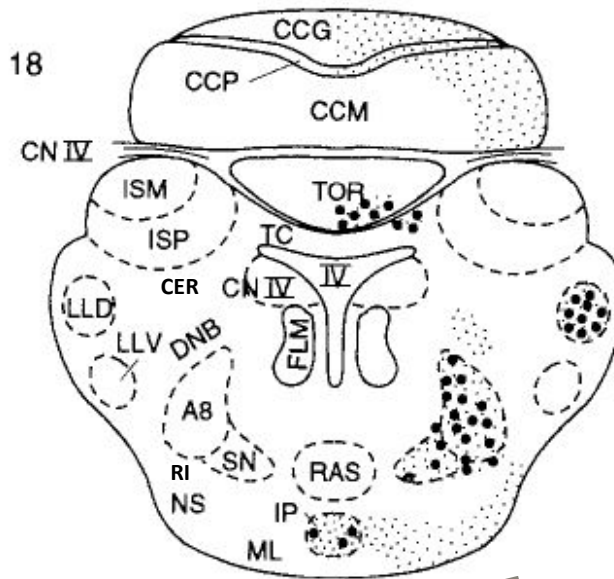


Figure 19: Naik 12, Lopez 18, CV 13, FS 13

5.14 Figure 19 Legend

A8 - area 8

CCG - granular layer of the cerebellar cortex

CCM - molecular layer of the cerebellar cortex

CCP - Purkinje layer of the cerebellar cortex

CER - locus ceruleus

CnIV - 4th cranial nerve

DNB - dorsal noradrenergic bundle

FLM - longitudinal medial fasciculus

IP - interpeduncular nucleus

ISM - magnocellular nucleus

ISP - parvocellular nucleus

LLD - dorsal nucleus of the lateral lemniscus

LLV - ventral nucleus of the lateral lemniscus

ML - lateral mammillary nucleus

NS - nigrostriatal tract

nIV - nucleus of the 4th cranial nerve

RAS - superior raphe nucleus

RI - reticular nucleus

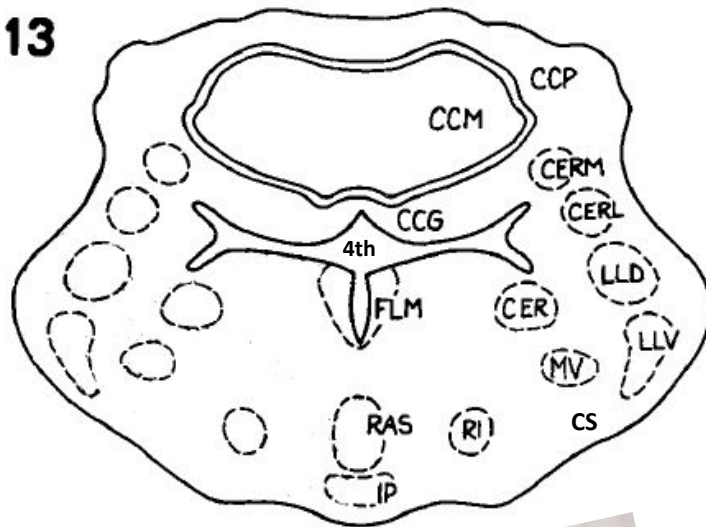
SN - substantia nigra

TC - tectal commissure

TOR - torus semicircularis

4th/IV - fourth ventricle

13



19

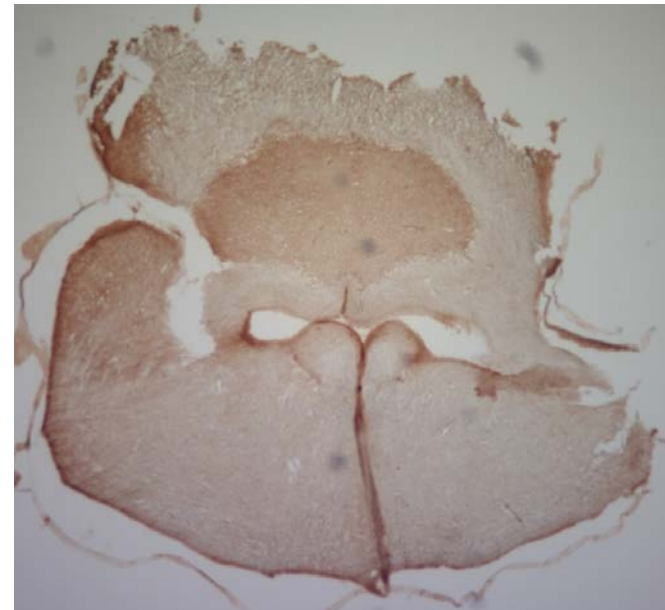
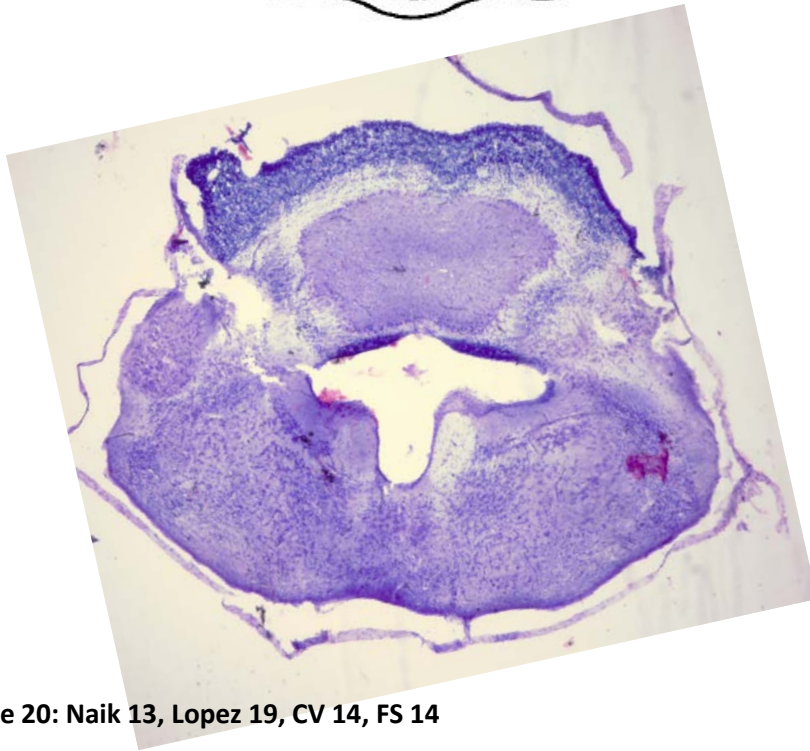
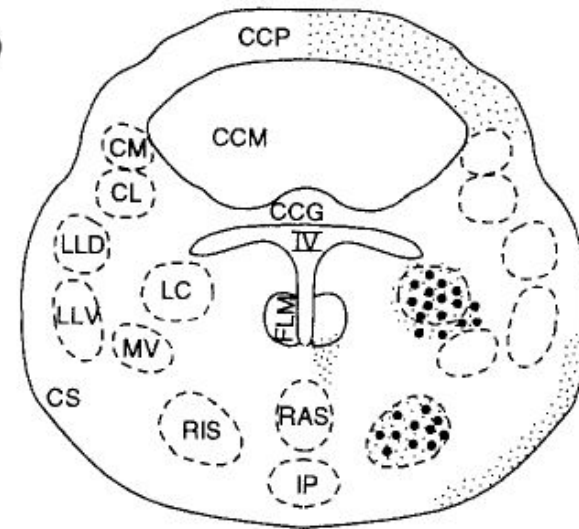


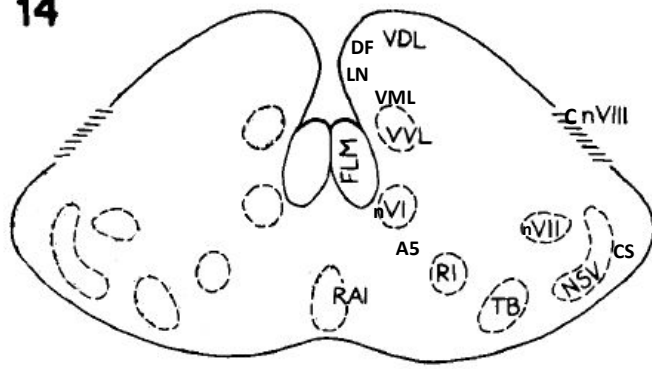
Figure 20: Naik 13, Lopez 19, CV 14, FS 14

5.15 Figure 20 Legend

CCG - granular layer of the cerebellar cortex
CCM - molecular layer of the cerebellar cortex
CCP - Purkinje layer of the cerebellar cortex
CER/LC - locus ceruleus
CERL/CL - lateral cerebellar nucleus
CERM/CM - medial cerebellar nucleus
CS - ceruleospinal tract
FLM - longitudinal medial fasciculus

IP - interpeduncular nucleus
LLD - dorsal nucleus of the lateral lemniscus
LLV - ventral nucleus of the lateral lemniscus
MV - motor nucleus of the trigeminal nerve
RAS - superior raphe nucleus
RI - reticular nucleus
RIS - superior reticular nucleus
4th/IV - fourth ventricle

14



20

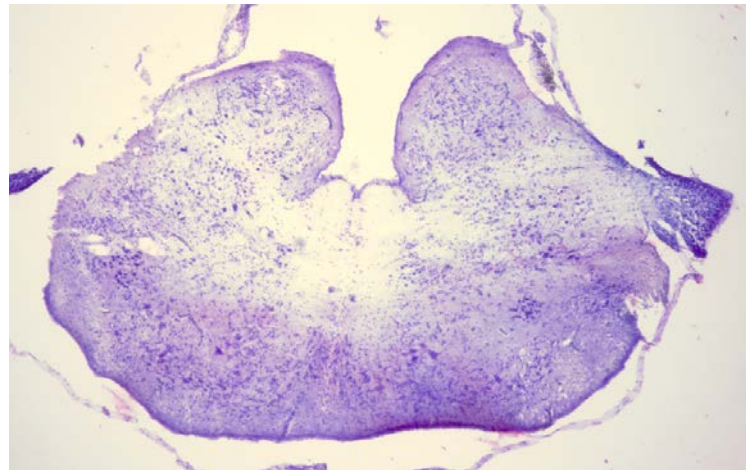
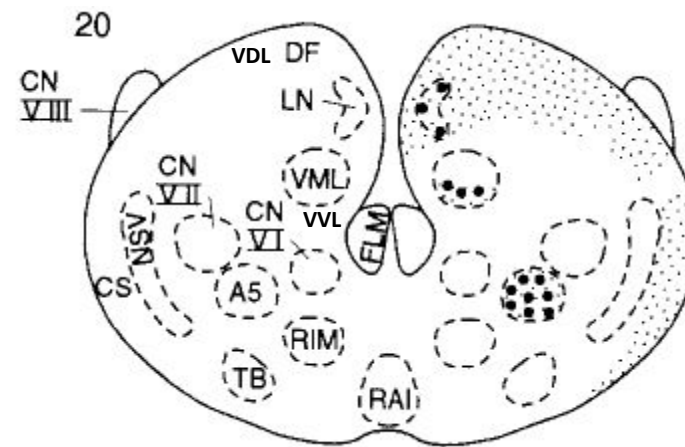


Figure 21: Naik 14, Lopez 20, CV 15

5.16 Figure 21 Legend

A5 - area 5

CnVI - 6th cranial nerve

CnVII - 7th cranial nerve

CnVIII - 8th cranial nerve

CS - ceruleospinal tract

DF - dorsal funiculus

FLM - longitudinal medial fasciculus

LN - laminar nucleus

NSV - spinal nucleus of the 5th cranial nerve

nVI - nucleus of the 6th cranial nerve

nVII - nucleus of the 7th cranial nerve

RAI - inferior raphe nucleus

RI - reticular nucleus

RIM - medial reticular nucleus

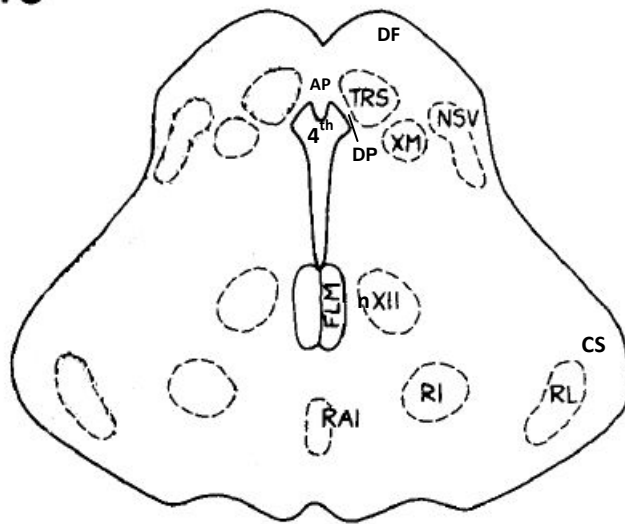
TB - nucleus of the trapezoid body

VDL - dorsal ventrolateral nucleus of the thalamus

VML - ventromedial vestibular nucleus

VVL - ventrolateral vestibular nucleus

15



21

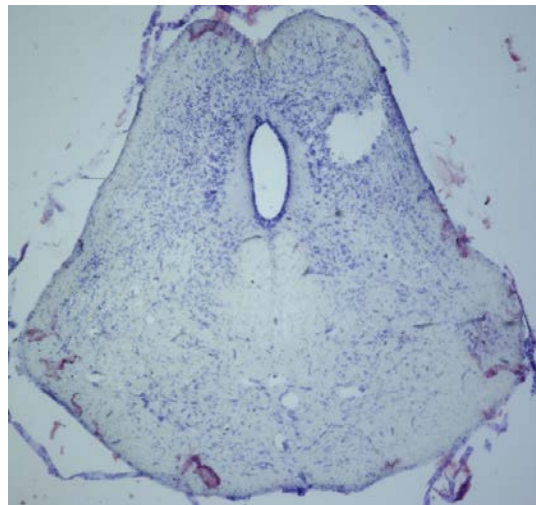
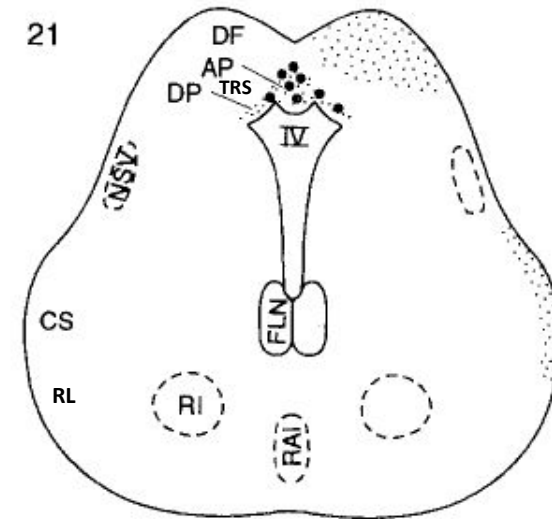


Figure 22: Naik 15, Lopez 21, CV 16

5.17 Figure 22 Legend

AP - area postrema

CS - ceruleospinal tract

DF - dorsal funiculus

DP - dorsal pretectal nucleus

FLM/FLN - longitudinal medial fasciculus

NSV - spinal nucleus of the 5th cranial nerve

nXII - nucleus of the 12th cranial nerve

RAI - inferior raphe nucleus

RI - reticular nucleus

RL - lateral reticular nucleus

TRS - nucleus of the solitary tract

XM - motor nucleus of the 10th cranial nerve

4th/IV - fourth ventricle

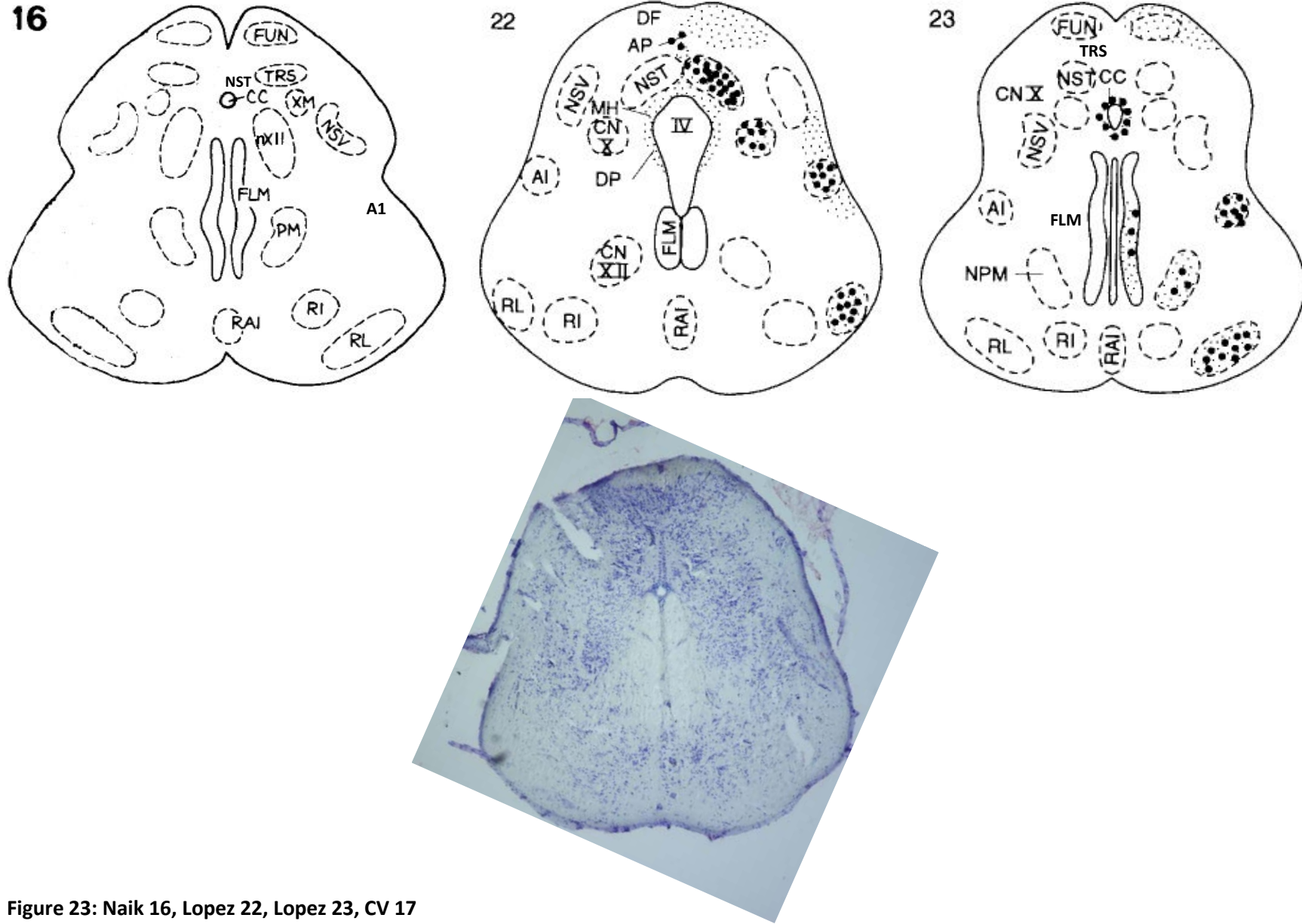


Figure 23: Naik 16, Lopez 22, Lopez 23, CV 17

5.18 Figure 23 Legend

A1 - area 1 of medulla

AP - area postrema

CC - central canal

CnX - 10th cranial nerve

CnXII - 12th cranial nerve

DF - dorsal funiculus

DP - dorsal periventricular fibers

FLM - longitudinal medial fasciculus

FUN - nucleus of the dorsal funiculus

IV - fourth ventricle

MH - medullohypothalamic tract

NPM/PM - nucleus profundus of the mesencephalon

NST - nigrostriatal tract

NSV - spinal nucleus of the 5th cranial nerve

nXII - nucleus of the 12th cranial nerve

RAI - inferior raphe nucleus

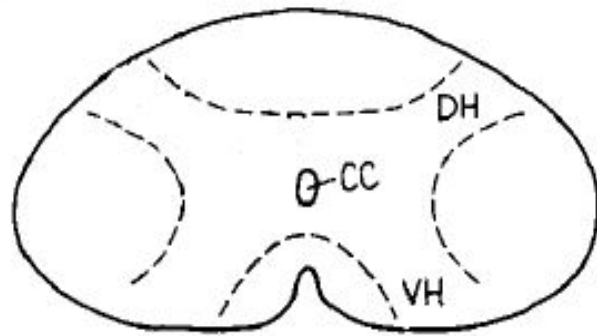
RI - reticular nucleus

RL - lateral reticular nucleus

TRS - nucleus of the solitary tract

XM - motor nucleus of the 10th cranial nerve

17



24

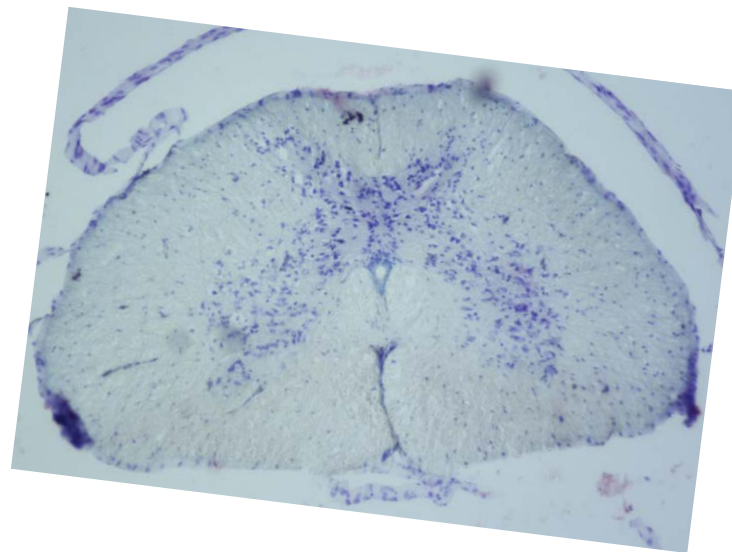
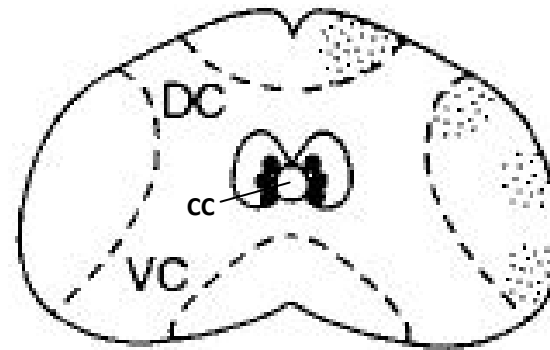


Figure 24: Naik 17, Lopez 24, CV 18

5.19 Figure 24 Legend

CC - central gray

DH/DC - dorsal horn of the spinal cord

VH/VC - ventral horn of the spinal cord

